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Selection of new eggplant (*Solanum melongena*, L.) lines

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Zusammenfassung

In den letzten Jahren ist das Interesse der Europäer am Verbrauch „Exotischer Gemüsearten“ gewachsen wie z.B. für asiatische Auberginengenotypen, die sich in der Farbe, im Geschmack und der Form von den standardmäßigen dunklen violetten Auberginenfrüchten unterscheiden. Diese Entwicklung kann die Einführung und die Kommerzialisierung neuer asiatischer Auberginensorten in Westeuropa beeinflussen und dadurch die Vielfalt des Angebots mit diesem Gemüse in den gemäßigten Regionen erhöhen. Aus diesem Grund bestand das Ziel dieser Arbeit darin, einige asiatische Genotypen (hauptsächlich vietnamesischen Ursprung) hinsichtlich ihres Wachstums und Ertrags zu untersuchen und diese mit typischen „europäischen“ Sorten bei einer Kultivierung im Gewächshaus zu vergleichen. Außerdem sollte geprüft werden, ob die genetische Variabilität von Samenherkünften verwendet werden könnte, um Klone zu selektieren, die den Wachstumsbedingungen in den Gewächshäusern unter den Klimabedingungen in Deutschland angepasst sind. In den *in vitro* Experimenten ist die Reaktion von 5 Auberginengenotypen auf die Mikrovermehrung und auf die *in vitro* Verfahren ausgewertet worden. Zusätzlich wurden die Einflüsse von Wachstumsregulatoren, NAA und 2.4D, auf die Kallus- und Organbildung und indirekte Pflanzenregeneration *in vitro* untersucht, um Aussagen hinsichtlich der Nutzbarkeit von biotechnologischen Züchtungsmethoden, zu erhalten. Das *in vitro* Klonen wurde mit dem Ziel durchgeführt, Klone von ausgewählten 5 asiatischen Auberginengenotypen zu erhalten, um Aussagen zu ihrer vegetativen und generativen Entwicklung sowie dem Ertrag zu erhalten, und mit typischen „europäischen“ Sorten unter Gewächshausbedingungen zu vergleichen. Alle Genotypen wurden einer der hydroponischen Substratkultur im Gewächshaus kultiviert. Die Entwicklung der Genotypen in organischen und mineralischen Substrat wurde verglichen. Das organische Substrat hat zum besseren Wachstum aller Auberginengenotypen geführt. Die aus der *in vitro* Vermehrung erhaltenen Klone der asiatischen Auberginengenotypen entwickelten sich zu normalen Pflanzen, die einen typischen Habitus erreichten und teilweise einen Ertrag hatten, der mit dem der Kontrolle vergleichbar war. Die besten asiatischen Auberginenklone hatten ähnliche Ergebnisse wie die der Kontrolle sowohl im Stadium des Blühbeginns, als auch im Ertrag. Ein wichtiges Merkmal für die Auswahl einiger asiatischer Klone war ihre Fähigkeit frühzeitig zu blühen, vergleichbar mit den „europäischen“ Sorten. Die asiatischen Genotypen waren charakterisiert durch schmalere und leichtere Früchte mit einem guten Geschmack, weniger Samen und einer guten Konsistenz des Fruchtfleisches im Vergleich mit den „europäischen“ Sorten. Ausgehend von den Ergebnissen die in den Untersuchungen erzielt wurden, scheint es möglich, neue stabile Genotypen zu erhalten, die geeignet sind für

eine Kultivierung in der „Substratkultur“ im Gewächshäusern und die ein Potenzial für den Erwerbsanbau unter europäischen Bedingungen haben. Für eine Optimierung der Wachstumsbedingungen, insbesondere das Formierungssystem, sind weitere Forschungsarbeiten erforderlich zumal diese Genotypen sehr starkwüchsig sind. Des Weiteren sollte der optimale Erntezeitpunkt und die Fruchtqualität untersucht werden.
Schlagwörter:
Aubergine, Substratkultur, Fruchtqualität, Blühzeitpunkt

Abstract

Recently, there has been a growing interest of the Europeans in the consumption of „exotic vegetables“ like those of Asian eggplant genotypes different in colour, taste and shape from the traditional dark violet eggplant fruits. This may influence the introduction and commercialisation of Asian eggplant types in Western Europe, which will contribute to increase the biodiversity of this crop in temperate regions. Therefore, this work aimed at screening 4 Asian eggplant genotypes (mainly of Vietnamese origin) concerning their growth and yield in greenhouses in comparison to „European“ breeds. Moreover, it should be tested whether the genetic variability of seed progenies could be used to select plants adapted to the growing conditions in greenhouses under the climatic peculiarities in Germany. In *in-vitro* experiments the response of 5 selected eggplant genotypes to micropropagation and *in-vitro* manipulation has been evaluated. Further on the influence of plant growth regulators, e.g. NAA and 2,4 D, on the callus and organ formation, and indirect plant regeneration *in-vitro* were studied in preparation of use of biotechnological breeding methods later on. The *in-vitro* cloning was carried out with the aim to produce clones of 5 Asian eggplant genotypes to evaluate their vegetative and generative development, as well as the yield, in comparison with typical „European“ varieties under greenhouse conditions. All genotypes were cultivated in „substrate culture“ with drip irrigation. The influence of organic and mineral substrates on the growth and development of the eggplant genotypes was compared. The organic substrate favoured better growth of all eggplant genotypes. The *in-vitro* derived clones of Asian eggplant genotypes developed to normal plants that reached full maturity and some of them had a yield comparable with that of the controls, the typical „European“ varieties. The best Asian eggplant clones gave similar results as the controls in the beginning of flowering and the yield. The early flowering feature characteristic of some clones of Asian origin comparable to that of the typical „European“ varieties is very important for selection. Asian genotypes were characterized by smaller and lighter fruits having good taste, less seeds and good consistency in comparison with the „European“ varieties used as control. Derived from the results obtained in this research, it seems possible to obtain new stable genotypes for „substrate culture“ system in greenhouse with a potential for commercial production under European conditions. The optimization of growing conditions especially the pruning system needs further research for these very vigorously growing plant types. Further on studies regarding optimal harvesting time and fruit quality shall be done.

Keywords:

Eggplant, substrate culture, fruit quality, flowering

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Dedicated To...

… my family, who offered me unconditional love and support throughout the course of my studies,

and

special thanks to MS for his love and patience.

Abbreviations

AVRDC	Asian Vegetable Research and Development Centre
BA	6-Benzylaminopurine
CIM	Callus Induction Medium
DNA	Deoxyribonucleic acid
EGGNET	Eggplant Genetic Resources Network
EPA	Environmental Protection Agency
FAOSTAT	FAO Statistical Database
FFTC	Food and Fertilizer Technology Centre
GRIN	Germplasm Resources Information Network's
IAA	Indole-3-acetic acid
IBPGR	International Board for Plant Genetic Resources
IPCC	Intergovernmental Panel on Climate Change
IPGRI	International Plant Genetic Resources Institute
MS	Murashige and Skoog plant growth medium
NAA	1-Naphthaleneacetic acid
NOA	B-Naphtoxyacetic Acid
NSWDPI	University of New South Wales Department of Primary Industry
PCR	Polymerase Chain Reaction
PGR	Plant Growth Regulator
RAPD	Random Amplification of Polymorphic DNA
TDZ	Thidiazuron
USDA	United States Department of Agriculture
ZMP	Zentrale Markt-und Preisberichtstelle GmbH
2,4 D	2,4-Dichlorophenoxyacetic acid

1 Introduction

Recently, there has been a growing interest of the Europeans in the consumption of "exotic vegetables" like those of Asian eggplant genotypes with different colour, taste and shape than the traditional dark violet eggplant fruits. Besides, the Asian communities in Europe still prefer their traditional vegetables. Those facts may influence the introduction and commercialisation of Asian eggplant cultivars in Western Europe, which will contribute to increase the biodiversity of this crop in temperate regions. Eggplant (*Solanum melongena*, L), also known as aubergine, melongena and brinjal (Lester and Hasan, 1991; Lawande and Chavan, 1998; EGGNET, 2005) is one of the most distributed and cultivated species of the *Solanaceae* family around the world (EGGNET, 2005). It belongs to the genus *Solanum*, which comprises about 1500 species (D'Arcy, 1991). There is a wide genetic diversity among the species within this genus; therefore they are very interesting for breeding programmes of the economically more important solanaceous crops such as eggplant. Eggplant is in the 4th rank of vegetables crops and staple food in many Asian, African and Mediterranean countries (FAOSTAT, 2005; Collonnier *et al*., 2001). The production of this crop is extended over all tropical and subtropical countries, and more than the half of its estimated world production comes from South East Asia (Prinz, 1989; FAOSTAT, 2005). Eggplant is a high-yielding crop (Lawande and Chavan, 1998). Nevertheless, an important feature of its yield is that it is partly dependent on environmental conditions (Saito and Ito, 1973; Wang *et al*., 1980; Sun *et al*., 1990; Abney and Russo, 1997). It is considered a vegetable of hot climates (Lawande and Chavan, 1998; Prinz, 1989), yet high relative humidity, low night air temperature and insufficient light are detrimental to eggplant fruit set (Saito and Ito, 1973; Wang *et al*., 1980; Sun *et al*., 1990; Abney and Russo, 1997). In Germany, as in all temperate regions, commercial production of eggplants can only be achieved in greenhouses. Therefore, it is of first importance to know the ecology of this crop in its centres of origin, in order to use appropriate cultivation systems and to optimize the growing conditions. The introduction of hybrids varieties of eggplant has been very successful in recent years, mainly, because of their high yield; a phenomenon that has caused in parallel, the degradation of the genetic resources of this crop (Collonnier *et al*., 2001). In 1997, aubergine was added to the list of species having priority for resources genetic preservation (Lester and Hasan, 1991; Daunay *et al*., 1998; Collonnier *et al*., 2001). In the case of eggplant, the erosion of its biodiversity results in the loss of many desirable agronomic features like that of resistance to abiotic stress (Baksh and Iqbal, 1979).

The introgression, through conventional breeding and biotechnological approaches, of total or

high resistance to diseases into the cultivated eggplant varieties is greatly needed (Lester and Hasan, 1991; Collonnier *et al*., 2001). Therefore, the use of cultivars from the eggplant centres of origin is of great importance for selection purposes, the collection of local accessions or landraces is urgent, and material should be screened for disease and pest resistance (Collonnier *et al*., 2001). Furthermore, association of yield with its contributing traits is important for making selection in the breeding programme (Bora and Shadeque, 1992). The wide genetic diversity of eggplant (Lal, 1991), in the cultivated and wild genotypes (Kashyap *et al*., 2003) gives an idea of its potential for genetic marker studies and selection work (Collonnier *et al*., 2001), and much can still be expected from traditional cultivars of eggplant and related genotypes (Sutarno *et al*., 1994). Eggplant breeding can be improved if the genetic richness of South Asian cultivars is better exploited in the future (Sutarno *et al*., 1993). Thus, the use of Asian eggplant genotypes is of great value in the planning of breeding programmes. Likewise, Asian cultivars are most interesting for the selection of new eggplant genotypes adapted to greenhouse cultivation systems in middle Europe. Additionally, the selection of new stable genotypes for greenhouse cultivation can ensure higher quality, more diversity and resistance of the final product. Potentially, the production of these "exotic vegetables" in the temperate regions of Europe under greenhouse conditions will become another source of revenue for the grower without necessarily resulting in higher prices for the consumer, avoiding at the same time transportation costs and ensuring European production standards for this vegetable crop. Therefore, this work aimed at screening some Asian cultivars (mainly of Vietnamese origin) concerning their growth and yield in greenhouses, in comparison to that of European breeds. Moreover, it should be tested if the genetic variability of seed progenies could be used to select plants adapted to the growing conditions in greenhouses under the climate peculiarities in Germany.

2 Literature review

2.1 Economical importance, world production and commercialisation of eggplant

The best known eggplant species is *Solanum melongena* (brinjal, eggplant or aubergine), widely cultivated in Asia (78 % of world production) and to a lesser but still important extent in the Mediterranean basin, including Turkey (about 19 % of world production) (EGGNET, 2005). Eggplant is the third most important crop in the *Solanaceae*, after potato and tomato (FAOSTAT, 2000). The world production of eggplant increased from 18 million Mt in 1995 to 30.5 million Mt in 2005 (FAOSTAT, 2005), being Asian and Mediterranean countries among the major producers (Table 2-1).

2.1.1 Field cultivation of eggplant

According to FAO statistics between 2002 and 2004, the Mediterranean countries members of the EU and major producers of this crop, tend to stabilize or slightly decrease the physical area (ha) dedicated to the production of aubergines without affecting the final yield (t/ha). Paralelly, it is Egypt and Turkey, respectively, that produce more eggplant than all of Europe. Nevertheless, Spain (45.31 t/ha) and Italy (28.52 t/ha in average) are the countries that have the highest yield in this region (Table 2-1), probably due to the use of tunnels as a complementary cultivation system. That ensures higher harvest than the traditional open field cultivation systems used in the other countries of this geographic area. Recently, both the production (t) and the acreage (ha) dedicated to the production of this vegetable increased about 100,000 ha in China, the worldwide biggest producer of the crop. As a result, the Chinese growers got about 1,000 000 t more of eggplant in 2004 than what was produced both in 2002 and 2003. Nevertheless, the production quantity (t) and acreage (ha) of eggplant obtained by the growers around the globe did not vary considerably in recent years (Table 2-1). The USA contributes to a great extent with the world's eggplant production (61,000 t/year Table 2-1) and is among the 20 bigger producers of this crop (FAOSTAT, 2005). Most of the eggplant production in the United States is located in Florida under open field cultivation system, but the crop is also produced under protected cultivation. In both cases, the final product finds its destiny in the local and regional markets within the United States. Besides, the quality of the final product is generally much higher from greenhouse versus field-produced vegetables (Cantliffe and Vansickle, 2003). The "Black Beauty" (Cantliffe and Vansickle, 2003), "Florida Market" and "Long Purple" are among the most popular eggplants

grown in Florida, as well as “Thai Eggplant”, which is destined to the local ethnic market (Li *et al*., 2002).

2.1.2 Greenhouse and tunnel production system

The Netherlands with 449.35 t/ha in three years average, exhibits the highest yield of this crop under greenhouse cultivation conditions, followed by France (44.17 t/ha in average) with a combination of open field and protected cultivation systems (Table 2-1). Since 25 years ago, when protected crop cultivation first came into being, the surface of greenhouses has grown more than 2000 ha per year (FAOSTAT, 2002). Significant new greenhouse vegetable production that was transferred to commercial producers has been primarily responsible for dramatic yield increases over the last years (Greer and Diver, 2000). The Mediterranean countries of Europe have one of the largest concentrations of protected crop cultivation in the world with approximately 100,000 ha dedicated to vegetable production grown in greenhouses, and 300,000 ha grown with law tunnels and mulching, which has contributed to the increase in the eggplant production. Eggplants together with tomatoes, peppers, cucumbers, muskmelons and watermelons are the mayor crops grown under protected cultivation in this region (Cantliffe and Vansickle, 2003).

Table 2-1 Major producers of eggplant (FAOSTAT, 2008)

World Region	Country	2002			2003			2004		
		ha	t	t/ha	ha	t	t/ha	ha	t	t/ha
Medite-rranean	Spain	3691	154,412	41.83	3876	175,629	45.31	3852	175,534	45.57
	Italy	12175	332,449	27.31	12881	368,992	28.65	12373	366,461	29.62
	Turkey	37000	955,000	25.81	36000	935,000	25.97	35000	900,000	25.71
	Egypt	36122	82,6870	22.89	43410	1026,353	23.64	43151	1046,742	24.26
	Greece	3200	77,000	24.06	3300	74,000	22.42	3009	72,547	24.11
Europe	Netherland	77	33,000	428.6	85	39,000	458.8	89	41,000	460.7
	France	501	20,234	40.39	494	19,823	40.13	475	20,292	42.72
North America & Orient	USA	2100	61,000	29.05	2100	72,544	34.55	2100	68,493	32.62
	China	823723	15433,284	18.74	851627	16029,929	18.82	901572	16530,287	18.34
	India	500000	8350,000	16.70	500000	7830,000	15.66	516400	8477,300	16.42

In Europe, there is a tendency to diversification in the eggplant market. Consumers show interest in "exotic" cultivars with different colour, shape, size and flavour than those of the traditionally commercialised dark violet or lilac oblong eggplant berries.

For the time being nevertheless, the most common varieties in the European market are High Yield Varieties ranging from the classical dark lilac coloured and oblong shaped fruits to the lighter lavender or pink and slender ones.

Eggplants are of considerable economic importance (Fig. 2-1) in some parts of Europe as vegetables, but they also obtain interest throughout Europe from breeders, seed companies, growers, consumers and phytochemists, who are concerned with better use of the genetic resources of eggplant (EGGNET, 2005).

As shown in (Fig. 2-1) the consumption of eggplant in Western Europe has been increasing recently. The quantity of the crop imported into the EU western member countries has been somewhat stable between 5 and 6 Mt in the last three years. However, the exported amount of this vegetable from EU members to the others has steady increased from 7 to 10 Mt.

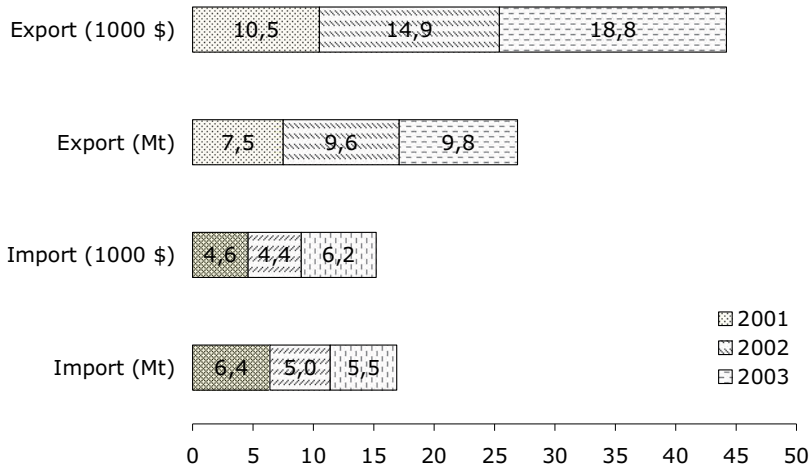


Fig. 2-1 Trade of eggplant within the EU (FAOSTAT, 2005)

Considering that the consumption of this crop per country is rather stable, the increase in exports means that there are more countries demanding this horticultural good.

The commercialisation of aubergines within the EU brings another source of income for the producers in the region (Fig. 2-1). At the same time it guarantees that the consumer receives a fresh and good quality commodity, which is produced locally, and hence prices are relatively fair.

The trade of this crop has generated a value of 10 to 18 thousands USD for concept of regional exports within the EU. Positively, the cost of importing it in the region has been kept around one thousand USD per Mt. Table 2-2 shows the German imports of this crop from the Dutch and Mediterranean markets.

Table 2-2 German import of eggplant in t (ZMP, 2006)

Delivering country	1999	2000	2001	2002	2003	2004
Netherlands	12,278	11,216	10,257	12,423	17,079	14,336
Spain	10,618	10,674	10,897	11,193	10,866	11,767
Italy	2,482	1,846	1,957	1,525	978,00	1,236
Turkey	905	1,694	2,524	1,276	1,232	1,789

The major exporters of eggplants to Germany are the Netherlands and Spain. Turkey has slightly increased the exports of aubergines to the German market, while Italy has done the contrary. Generally, the imports of this crop tend to increase, indicating higher consumption of eggplant in this country (Table 2-2).

The Netherlands produces eggplant in greenhouse substrate culture. Spain and Italy obtain most of their eggplant production from protected cultivation system, e.g. tunnel, mulching. Both greenhouse and protected cultivation ensure major quality and stability of the harvest (FAO, 2004; Bougoul *et al*., 2005). In the case of Turkey, also the production of crops, specifically vegetables like aubergines and tomatoes, as well as ornamentals, occur with more frequency under protected cultivation system (Cemek *et al*., 2005).

Table 2-3 Production of eggplant in South East Asia in (FAOSTAT, 2008)

Year / Parameter	2002	2003	2004	2005	2006	2007
Area Harvested (ha)	71672	76953	77894	78607	82262	86037
Production Quantity (t)	519528	545340	562433	589394	618276	656294
Yield (t/ha)	7,25	7,09	7,22	7,50	7,52	7,63

In Table 2-3 one can observe that there has been an increase in both, the cultivated area (ha) and the production (t) of this crop in South East Asia where eggplant has one of its biggest centres of origin and is an important agricultural product for the local and export markets.

That indicates a tendency to a higher interest in the commercialisation and consumption of this vegetable crop. Even though the production (Table 2-3) has increased in the area from 519528 in 2002 to 656294 t in 2007; the yield (~7 t/ha) still is lower than that in other geographical regions (see Table 2-1). That relatively low productivity of eggplant in this region, where diversity of this crop is high, suggests that the production conditions may need improvements in South East Asia. The low yield of aubergines in this area could be due to the wide use of hybrid varieties developed for intensive production, instead of cultivars bred for local ecological conditions.

2.2 Biological characterisation of eggplant

2.2.1 Origin

The eggplants are widely distributed in the continents of Asia, Africa and South America. The best known eggplant species is *Solanum melongena*, L also called aubergine or brinjal (EGGNET, 2005). Its biodiversity is wide in African and Asian countries (Daunay and Lester, 1988), but there has been noticeable genetic erosion in Indonesia, Thailand and Malaysia, due to the preferential use of high yielding varieties (Lester and Hasan, 1991; Collonnier *et al*., 2001). Nowadays, this solanaceous crop is more important in China, India, South East Asia, northern Africa and the Mediterranean area. Besides, it is considered one staple vegetable in many tropical countries, (Lawande and Chavan, 1998). Lester and Hasan (1991) and Lester and Daunay (2003) believe that the ancestors as well as the closest wild relatives of *S. melongena* probably originated in Savannah vegetation in the hilly equatorial regions of eastern Africa, where the diversity of topography, and hence climates and soils, led to the evolution of different species. *S. scabrum*, *S. macrocarpom* and *S. aethiopicum* are *Solanum* vegetables indigenous to Africa (Lester and Daunay, 2003).

2.2.2 Systematic

The classification of *Solanum*, in particular, began with Linnaeus (1753) in his "*Species Plantarum*", where he described the two species which are the corner stones of the eggplant complex, i.e. *S. incanum* and *S. melongena*. In later works, however, his concepts changed dramatically due to the high degree of morphological plasticity shown by these species. Other classifications (Table 2-4) followed in an attempt to unravel the confusion surrounding these species (Mace *et al*., 1999).

Table 2-4 Ancient taxonomical classification of *Solanum* (Mace *et al*., 1999)

Author	Achievement	Remark
Linnaeus "Species *Plantarum* (1753)"	Described *S. incanum* and *S. melongena.*	Led to confusion because of morphological plasticity of these species.
Dunal (1852)	Intended to give the exact number of African *Solanum* species.	-
Dammer (1915)	Increased the number of African *Solanum* species to 200.	Did not clarify the delimitations of these species.
Bitter (1923)	Begun to unravel the confusion surrounding the African *Solanum* species by using the species-aggregates concept.	Indicates close relationship between groups of species but does not force premature nomenclatural decisions for that group.

Bitter (1923) circumscribed three species-aggregates in his series *Incaniformia*, which encompassed the majority of his 81 taxa of the so-called *S. incanum* aggregate: namely, *S. campylacanthum* (Hochst.) *sensu ampliore* Bitter, *S. bojeri* (Dunal) *sensu ampliore* Bitter and *S. incanum* L. *sensu ampliore* Bitter. Bitter´s work, however, was ignored and his taxa were indiscriminatelly treated as one species, *S. incanum* (Mace *et al*., 1999). The modern recognition of distinct groups of taxa within series *Incaniformia* begun with Jaeger (1986), later modified by Lester and Hasan (1991) and Samuels (1996). The groupings (Table 2-5) described by Lester and Hasan (1991) have followers, like Sakata and Lester (1994) and Samuels (1996); as well as opponents, namely, Karihaloo and Gottlieb (1995), Karihaloo and Rai (1995) and Karihaloo *et al*. (1995).

Table 2-5 Taxonomical classification of *Solanum* according to centre of origin (Lester and Hasan, 1991; Mace *et al*., 1999)

Wild taxa of *S. incanum sensu lato*, from Africa			Weedy and cultivated taxa of *S. melongena*, from Asia		
Group A	*S. campylacanthum*	East and South Africa	Group E	*S. melongena (S. insanum)*	India
Group B	*S. panduriforme*	South Africa	Group F	*S. melongena (S. cumingii)*	S.E. Asia
Group C	*S. incanum*	North Africa, Arabia	Group G	*S. melongena (S. ovigerum)*	S.E. Asia
Group D	*S. lichtensteinii*	South Africa	Group H	*S. melongena (S. melongena)*	world-wide

The domestication of *Solanum* vegetables in Africa depended on the development of agricultural systems and the availability of suitable wild or introduced species (Lester and Daunay, 2003). Studies by Daunay *et al*., 2001 recognize three African vegetable *Solanum* species (Fig. 2-2), and *Solanum melongena* L., which was domesticated rather in South East Asia than in Africa, but whose close related wild species are indigenous in Tropical Africa and the crop is extensively grown in both northern and southern Africa.

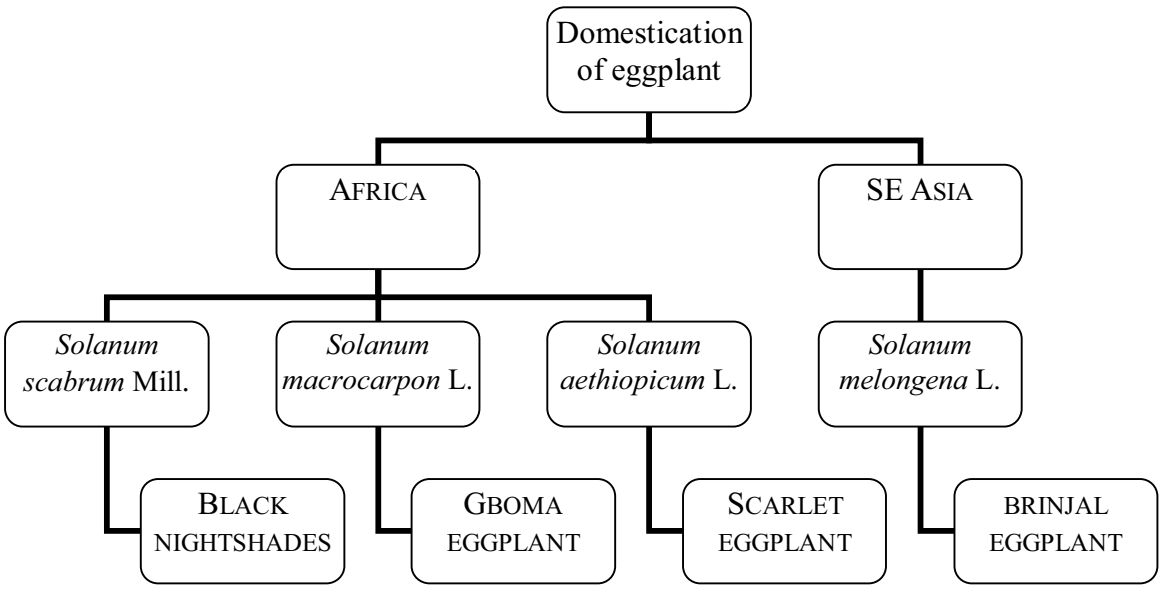


Fig. 2-2 The domestication of *Solanum* (Lester and Daunay, 2003)

There is a wide variability of the plant morphology, the colours, size, shape and taste of fruits, biochemical and physiological features, as well as the growing conditions requirements within eggplant. Based upon these differences, Filov (1958) classified five subspecies of *S. melongena* (Fig. 2-3).

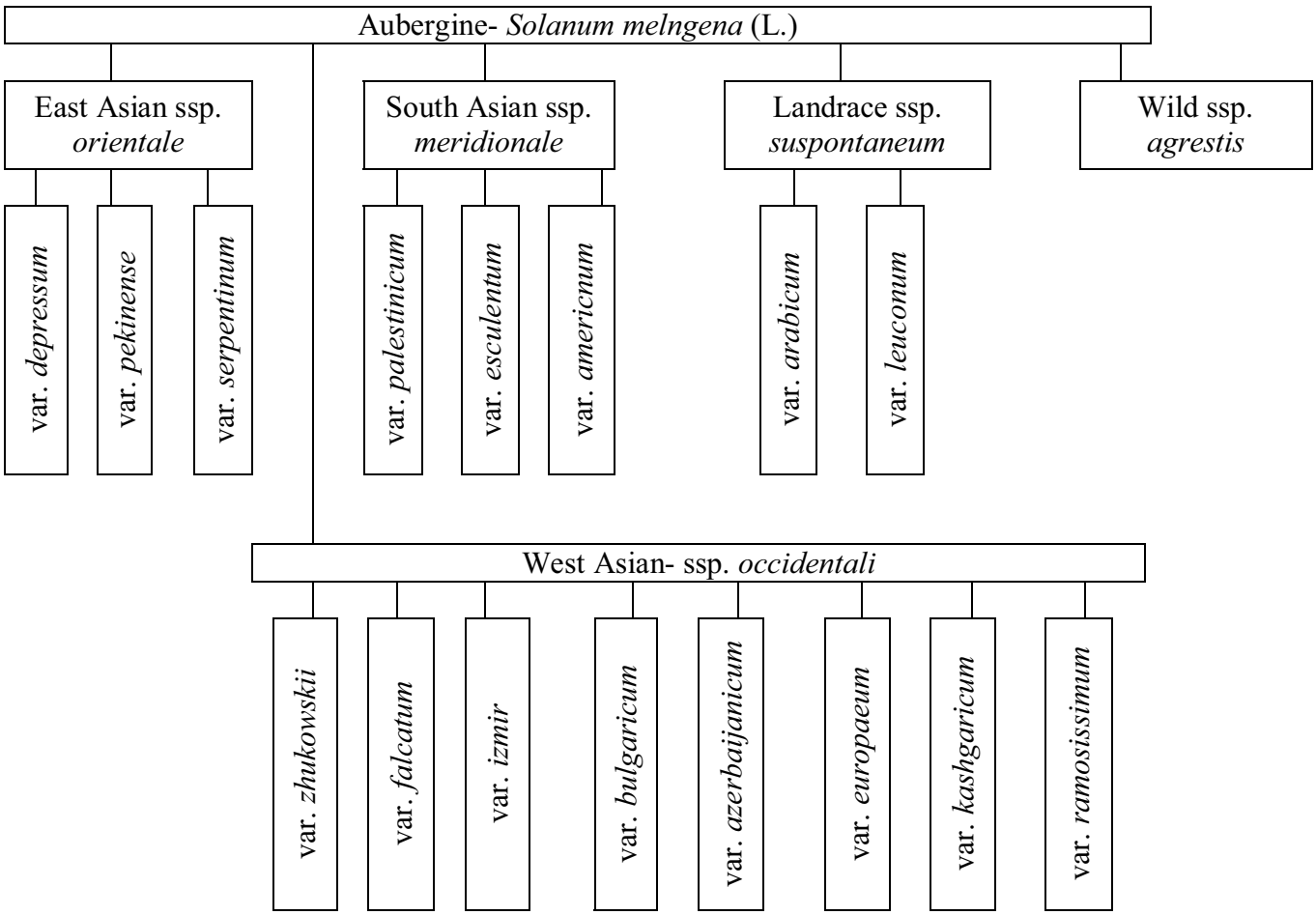


Fig. 2-3 Classification of eggplant according to morphology, physiology and climatic requirements (Filov, 1958)

Certainly, this biodiversity of eggplant could be used to select new types for greenhouse cultivation in Europe. We focused our work on eggplants from South East Asia, specifically from Vietnam. Landraces and cultivars from this country partly exhibit a high variability and seem to belong to the *Solanum melongena* ssp. *orientale* var. *pekinensis* and spp. *occidentalis* var. *falcatum* (Böhme *et al*., 2004a; b).

Taxonomy of eggplant (Lawande and Chavan, 1998; Mace *et al*., 1999; Collonnier *et al*., 2001).

Class	*Magnoliopsida*		
Family	*Solanaceae*	Genus	*Solanum*
Subfamily	*Solanoideae*	Subgenus	*Leptostemonum*
Tribe	*Solaneae*	Species	*melongena*

2.2.3 Description of the botanical characteristics

According to the botanical classification of eggplant, there are 3 major varieties of this crop under the species *melongena* (Lawande and Chavan, 1998):

S. melongena var. *serpentium* (Snake Aubergine)

S. melongena var. depressum (Dwarf Aubergine)

S. melongena var. *esculentum* (Common Aubergine)

S. melongena and its varieties are very diverse in fruit form and colour (Prinz, 1989; Lawande and Chavan, 1998). That variation is continuous, which means that the existing subclassifications into botanical varieties and subspecies have no practical value. More practical would be a classification into cultivar groups and cultivars. Worldwide, important cultivars are "Black Beauty", "Florida Market" and "Long Purple" (Lawande and Chavan, 1998); which belong to the botanical variety *esculentum*, as well as "RZ Adona" (Fig. 2-5) and "RZ Ritmo", which served as control in the experiments carried out for this study.

- Cultivars

Traditionally, genetic resources for vegetables production is kept in form of seeds from own garden or field production (Lester *et al*., 1990). Nowadays, there are commercial seed companies (Att.III) offering a wide range of eggplant varieties (Att.II) that supply farmers around the world with the necessary material for propagation of the crop. Most common eggplant cultivars (Table 2-6) for commercial use differ in earliness, as well as in size, shape and colour of mature fruit, and are F_1 hybrids (Granberry, 1990).

Table 2-6 Characteristics of most common eggplant cultivars (Granberry, 1990)

Eggplant cultivar	Days[1]	Type[2]	Plant Height (cm)	Fruit Colour	Fruit Shape
Beauty	65-67	F_1	63.5	Glossy black	Oval
Black Beauty	77-79	O.P.	61-76	Nearly black	Oval
Black Bell	68-72	F_1	71	Glossy, deep purple black	Round/oval
Black Jack	66-70	F_1	81	Medium dark	Oval
Dusky	61-64	F_1	61	Glossy black	Oval
Epic	63-65	F_1	91	Deep purple-black	Oval
Ichiban	57-59	F_1	81	Soft, dark purple	Slender cylindrical
Florida Market	84-86	O.P.	91-122	Glossy, very black	Tapered cylinder

[1]Expected no. of days from transplanting to maturity when grown under favourable conditions

[2]Type: F_1 = hybrid, O.P. = open pollinated

In South East Asia, local cultivars are more common than the high-yielding hybrid cultivars since they are better adapted to local conditions in despite of the fact that selection and registration of local cultivars still is rare. In this region there are two distinct types of eggplant cultivars, which may be distinguished as cultivar groups (Sutarno *et al*., 1993):

Cultivar Group Common Eggplant (Fig. 2-4). It is characterized by a robust habit, more or less pronounced purplish flowers, and persistent calices at the base of the big round to elongated oval fruits. The immature fruits are extremely variable in form, size and colour, showing variations between purple, green and white. A popular local type in Indonesia: "Kopek" and an "international" one: "Long Purple" belong to this cultivar group.

a)  b) 

Fig. 2-4 Fruits of cultivar Group Common Eggplant: a) cultivar "Kopek" (GRIN, 2005) and b) "Long Purple" (AgroHaitai LTD., 2005)

Cultivar Group Bogor Eggplant. It is characterized by a small spreading habit, small greyish leaves, and small greenish-white flowers. The fruits are round or flat-round, 4-10 cm in diameter, green near the calyx which partly envelops the fruit, and marbled-white at the top. Two popular local types in Indonesia: the tender, slightly-bitter taste "Kelapa" types for raw consumption, and the small sized fruits of "Gelatik" belong to this cultivar Group. Both cultivar groups do not cross easily, and the yielding capacity of cultivar group Bogor Eggplant is much lower than that of cultivar group Common Eggplant. On the other hand, cultivar group Common Eggplant is more susceptible to diseases and pests than cultivar group Bogor Eggplant (Sutarno *et al*., 1993).

2.2.4 Botanical description of eggplant

Common aubergine is an erect, branching, very polymorphous, perennial herb with stiff, hard stems up to 1.5 m tall (Fig. 2-5a), grown as an annual, and with strong, deeply penetrating tap root. All parts of the plants are covered with a grey *tomentum* and sometimes spiny, while the older ones may become woody (Sutarno *et al*., 1993; Tarragon Lane Ltd., 2005).

Leaves (Fig. 2-5b) are large, alternate and lobed with the underside of most cultivars (varieties) covered with dense wool-like hairs; and simple. The petiole is up to 10 cm long, the leaf-blade is ovate to ovate-oblong (3-25 cm X 5-15 cm), densely stellate, hairy, base rounded or cordate, often unequal, margin sinuately lobed, apex acute or obtuse (Sutarno *et al*., 1993; Tarragon Lane Ltd., 2005).

a)  b) 

Fig. 2-5 a) Stem and b) leaf of *Solanum melongena* var. *esculentum* cv. "RZ Adona"

Flowers grow as solitary or in 2 to 5 flowered clusters, opposite the leaves with 3-5 cm in diameter, they are hermaphrodite or male and star-shaped. The pedicel is 1-3 cm long, in the fruit up to 7 cm. The calyx is tubular-campanulate about 2 cm long with 5 to 7 lobes, spiny, woolly, persistent and enlarging in fruit where it often splits. The corolla is gamopetalous, deeply 5-6-lobed and stellating spreading, purplish-violet and heary beneath the lobes (Fig. 2-6a). The stamens (5-6) are prominent and converging into a cone about 1 cm long (Tarragon Lane Ltd., 2005). The anthers open by two terminal pores, the ovary is two lobular, the style simple and the stigma capitate (Sutarno *et al*., 1993; Tarragon Lane Ltd., 2005) (Fig. 2-6b). Eggplant does not have photoperiodic requirement for flower initiation. The solitary flowering types have very low flower drop frequency, while the clustered flowering genotypes may drop up to 80% of the flowers. The flower is usually perfect with functional male and female parts.

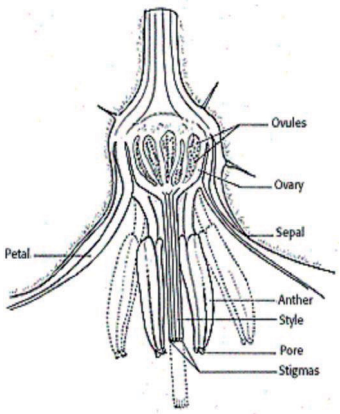
a)  b) 

Fig. 2-6 *Solanum melongena* var. *esculentum*: a) flower of cultivar "RZ Adona" (Arias, 2002) and b) the structure of eggplant´s flower (McGregor, 1976)

They may be long-styled flowers (stigma above or same level as stamen) or short-styled flowers (stigma below stamen), being the first more appreciated in number than the latest, because fruit set is higher for long-styled flowers compared with short-styled flowers (Chen et al., 2001). Usually, anthesis and pollen dehiscence in eggplant flowers occur between 6:00 and 11:00 in the morning, but they strongly depend on daylight, temperature and humidity, therefore, the exact timing for every area should be determined by observation and experience. Pollen viability is retained for 8–10 days at a temperature of 20–22 °C and with a relative humidity of 50–55% (Chen et al, 2001). Eggplant is a normally highly self-pollinated crop. The cone-like formation of anthers favours self pollination; but since the stigma ultimately projects beyond the anthers, there is an ample opportunity for cross-pollination. The rates of natural crosspollination may vary depending on genotype, location and insect activity. It has been reported that the extent of natural outcrossing was from 2 to 48% in eggplant varieties in India and from 3 to 7% in China. (Chen et al, 2001). The eggplant's fruits (Fig. 2-7) are like large pendent berries, that is fleshy, indehiscent, many seeded fruits containing no hard parts except the seeds, they vary in shape from oval to round, long to oblong and obovoid or subglobose to globose, up to 40 cm long and 20 cm in diameter but very variable (Allaby, 1992; Sutarno et al., 1993). The colour of the mature fruit varies from purple to purple-black, it can also be red, yellowish-white, white or green, shiny, yellow or mixed coloured (Allaby, 1992; Sutarno et al., 1993; Lindgren, 1996).

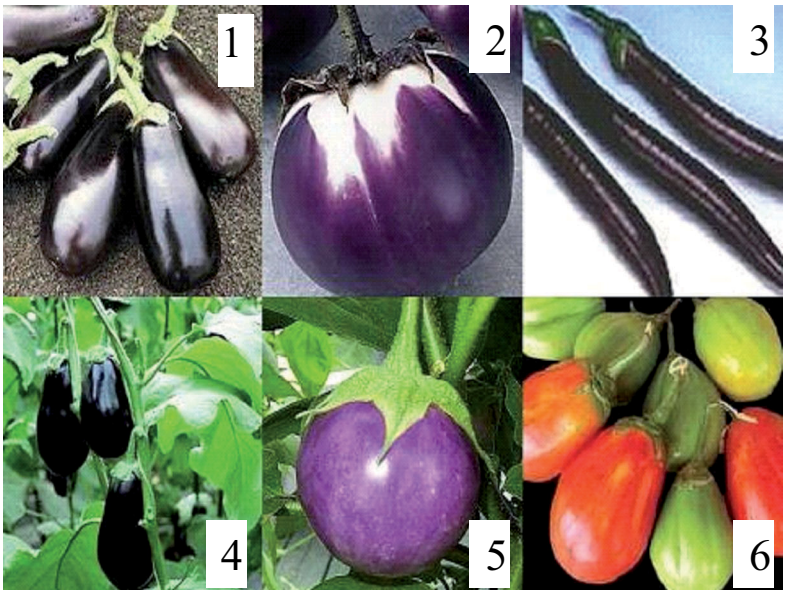


Fig. 2-7 The fruit of *Solanum melongena* var. *esculentum* is very variable: cultivars 1 "Bakota"; 2 "Blue Marble"; 3 "Slim Purple"; 4 "Classic"; 5 "Lao Lavender"; 6 "Comprido Verde Claro" (Standart out inc, 2005)

Fig. 2-8 Seeds from eggplant (Böhme, 2001)

Seeds (Fig. 2-8) are numerous; small and light brown. The eggplant seeds grow buried in the fruit´s flesh; they are round-flat with a diameter of 2 to 4 mm and 0.5 to 1 mm thick. The 1000 seed weight amounts to 4-5 g (Sutarno *et al*., 1993)

2.3 Nutritional value and marketable quality of eggplants fruits

Eggplant fruits have a reasonable nutritional value and can be compared with the value of tomato (Sutarno *et al*., 1993). The chemical composition (Table 2-7) and the texture of eggfruits make them attractive for human consumption all over the world. The young and almost mature fruits are used as vegetable, they may be roasted, fried, stuffed, cooked as curry, pickled or prepared in some other manner (Lindgren, 1996); in Indonesia and Malaysia they are also eaten raw (Sutarno *et al*., 1993). Eggplants in the broad IPGRI sense encompass

several species of *Solanum* that are grown as gastronomically interesting and nutritionally valuable vegetables, also as desert fruits and medicinal plants, as well as many related wild species. Also, some *Solanum* species are rich sources of different kinds of steroidal alkaloids and saponins, which have a great interest for pharmaceutical research (EGGNET, 2005). Brinjal eggplant is known to have medicinal properties (Lawande and Chavan, 1998), it is widely use in traditional medicine against haemorrhoids, ulceration, diabetes, asthma, cholera, bronchitis, dysuria, high blood cholesterol level, otitis and toothache (Sutarno *et al*., 1993).

Table 2-7 Chemical composition of eggplant* (Lawande and Chavan, 1998)

Constituent	Content	Constituent	Content
Oxalic acid	18 mg	Sodium	3,0 mg
Calcium	18 mg	Copper	0,17 mg
Magnesium	16 mg	Potassium	2,0 mg
Phosphorus	47 mg	Sulphur	44 mg
Iron	0,9 mg	Chlorine	52 mg
Moisture content	92,7 %	Vitamin A	124 IU
Carbohydrates	4,0 %	Thiamine	0,4 mg
Protein	1,4 %	Riboflavin	0,11 mg
Fat	0,3 %	ß-Carotene	0,74 µg
Fibre	1,3 %	Vitamin C	12 mg
Energy	24 kcal	*per 100 g edible portion from diff. eggplant cultivars	

Despite the great similarity in the chemical composition of eggplant cultivars (López-Andréu *et al*., 1992), there are differences that are typical for distinct cultivar groups. The physical characteristics of fruits, e.g. shape, colour, presence of spines on the calyx or foliage colour, were found to influence the chemical composition (Dighe, 1995). Other factors like culture methods, soil water availability, irrigation and fertilization may also affect the mineral content of eggplant´s fruit (Russo, 1995). Bajaj *et al*. (1979) found that the long- fruited cultivars contain, on average, large amounts of dry matter, amino protein, total water-soluble sugars, free reducing sugars, anthocyanins, phenols, and glycoalkaloid as solanin. The percentages of nitrogen and ether extract values were similar for purple, green and white eggplant cultivars (Dighe, 1995). However, the white cultivars contained twice as much crude fiber as the purple and the green cultivars (Sherman, 1974; Bajaj *et al*., 1979; Dighe, 1995); while the amino acid contents were higher in the purple cultivars and lowest in the white (Flick *et al*., 1978). The enzymatic activity, anthocyanin and glycoalkaloid contents as well as the organic and mineral

elements of purple, green and white eggplant cultivars have been analysed. Polyphenoloxidase activity seemed to be correlated to the amount of copper present in the eggfruit and was found to be highest in purple, lower in green and lowest in white cultivars. The iron content, apparently, had influenced the catalase activity for green, purple and white eggplant varieties in decreasing order, while zinc had correlated positively with alcohol dehydrogenase activity, which was determined to be higher in white than in purple cultivars. Also peroxidase activity was found to be higher in the white than in the other two cultivars (Ramaswamy and Rege, 1977a,b; Flick *et al*., 1978; Sidhu *et al*., 1982). Anthocyanin is responsible for the great variety of colours of the eggplant´s fruit and its content varies widely among different eggplant cultivars. The white cultivars, independently of the fruit shape, e.g. long, round, have been found to lack this pigment (Bajaj *et al*., 1979 and Sidhu *et al*., 1982). The presence of glycoalkaloids, which occur often among members of *Solanacea* family, are responsible for bitterness in eggplant fruits and its high levels (20mg/100g fresh weight) produce bitter taste and off-flavour. Potassium, chlorine, magnesium and calcium are present in high concentrations in all three cultivars of eggplants. Nevertheless, potassium and chlorine are highest in the green and lowest in the purple cultivars (Flick *et al*., 1978; Bajaj *et al*., 1990).

- EU Marketing Standards for Fruit and Vegetables (EU-Regulation)

Imports into the EU of fresh fruit and vegetables are checked for compliance with EU-harmonized marketing standards. These standards apply at all marketing stages and include criteria such as quality, size, labelling, packaging and presentation. Marketing standards (Att. VI) apply to produce intended for the fresh market. A conformity certificate is required for all shipments of fresh produce destined for the fresh market before release into free circulation within the EU, according to Regulation 408/2003 (amendment to reg. 1148/2001).

Related reports from USEU Brussels:

Report number	Title	Date released
E21134	Horticultural Products Certification	11/9/2001
E21058	EU Fruit & Vegetables Regime	5/7/2001
E22084	Food & Agricultural Import Regulations & Standards	8/21/2002

Eggplant exhibits wide variability in fruit shape and colour. For the standard fruit type (large, dark purple, pear like) there are quality regulations by the EU; which are very general referring to external characters (e.g. diameter, length and weight) of the fruits (EU Council Regulation, 1996/2005). According to this regulation, minimal values for the diameter should be 40 mm, for the length 70 mm and for the weight 100 g. Additional quality requirements are mentioned as without overdevelopment of seeds and no defects of colouring. For other fruit

types as produced in Asian countries (smaller, oblongoid or round) there are no regulations at the moment. Harvesting time is determined empirically by the grower assessing the firmness and the brightness of fruit skin (Böhme *et al*., 2006; Böhme *et al*., 2008). The European Regulation for quality standards on eggplants refers to the aubergine cultivars that are large, dark purple and pear shaped, which can not be applied to other eggplant types with different size, colour and shape. Therefore, there shall be another way of evaluating postharvest quality of new or "exotic" eggplant types for commercialisation within the EU. Alternatively to the existing EU regulation for quality standards of this crop, could be the use of non-destructive methods for the evaluation of eggplant´s fruit maturity. Böhme *et al*., (2008) studied different parameters to assess maturity of distinct fruit types. Maturation could be described with different parameters, e.g. dry matter content, optimal maturity index (destructive method), as well as colour evaluation or elasticity module (non-destructive method). There seem to be, however, a correlation between the tested non-destructive methods and the eggplant genotype. Therefore, the most appropriate parameter has to be found as reference quantity for each fruit type.

2.4 Review about breeding methods applied to eggplant

Often, long periods of vegetative growth elapse before the agronomical evaluation of crops can take place. Traditional cultivar identification based on morphological traits requires extensive observation of mature plants, and in many cases, it lacks definition and objectivity; furthermore, the development of cultivar-specific genetic markers is desirable for cultivar identification and protection (Yang and Quiros, 1993). Thanks to the development of molecular biology (Yang and Quiros, 1993) in recent times, and the improvement of bio-techniques, both time and resources are saved, as well as a more precise characterization of genetic traits is achieved. The use of molecular techniques might be employed for identification, measurement of the genetic variation, establishment of genetic relationships and correlation of markers with desirable traits for introgression (Kresovich *et al*., 1992). Molecular tools also assist scientists to better understand the distribution of pathogens and identify genetic sources of resistance to these pathogens. For example in tomato, resistant lines to leaf curl viruses have been obtained using a series of molecular-based tools to efficiently develop resistant varieties from wild tomato germplasm (Samson, 2001). Different studies demonstrated the usefulness of molecular biology techniques, e. g. AFLP, Microsatellites and RAPD-PCR, to obtain genetic markers for the identification and taxonomy of different species, as well as to assess the genetic diversity among individuals.

2.4.1 Amplification fragment length polymorphism (AFLP)

AFLP was developed by Vos *et al*. (1995), as a new DNA marker system combining the advantages of restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) cited in Nunome *et al*. (2001). It was described as efficient in generating a large number of genetic markers in a single experiment according to Powell *et al*. (1996b) and Jones *et al*. (1997); and suitable for the construction of high-density maps and for the positional cloning of interested genes, by Thomas *et al*. (1995), as well as a robust tool for detecting genetic diversity and determining genetic relationships (Lee *et al*., 2002) within and among a group of *Solanum* species (Mace *et al*., 1999). The rapid and easy assays with AFLP markers is a significant advantage for breeding purposes (Nunome *et al*., 2001), especially in the eggplant where the frequency of DNA polymorphism is very low (Karihaloo *et al*., 1995; Nunome *et al*., 2001). However it is difficult to transfer the data from dominant AFLP markers to other segregating populations, which can be overcome by converting a marker framework covering the entire map into SCAR or CAPS (Paran and Michelmore, 1993; Konieczny and Ausubel, 1993, respectively) cited in Nunome *et al*. (2001) and RFLP (Nunome *et al*., 2001).

2.4.2 Microsatellites or single sequence repeats (SSR)

Microsatellites are one of the choices to obtain DNA-based markers and the construction of genetic linkage maps. They are highly polymorphic, multi-allelic, frequently codominant, PCR-based, easily reproducible and randomly and widely distributed along the genome (Powell *et al*., 1996a). Microsatellites markers have been described as very informative, stable, inexpensive and, generally, developed in three ways: by transfer from closely related species, by searching in sequence databases and by screening of DNA or small insert libraries, or enrichment libraries of microsatellites with tandem repeated oligonucleotide probes and sequencing of candidates clones (Provan *et al*., 1996; Bell and Ecker, 1994; Powell *et al*., 1996a, respectively) cited in Nunome *et al*. (2003). In spite of their many advantages for genetic analysis and marker assisted selection, not all microsatellites PCR primer pairs designed for one organism are applicable across genera, due to high variation in flanking sequences of microsatellites between the donor and the receptor, and the frequent loss of the microsatellite motifs where amplification occurs. Transfers between potato and tomato, and from tomato to eggplant confirm it. Even though, it is possible to develop microsatellite markers by searching genome sequences databases, it only works for some plant species. In eggplant, out of 38 sequences available in the Genebank none contains microsatellites. The library screening method is a reliable way of finding microsatellites in species for which little

genomic information is available because, if thought, screening of enriched libraries of microsatellites clones is an efficient method for isolating useful markers, it shows a high rate of redundant clone identification (Nunome *et al*., 2003). Identified microsatellites loci were distributed throughout the linkage map of eggplant, as well as for rice, barley, maize, *Arabidopsis* and soybean cited by Nunome *et al*. (2003). Clustering of microsatellites, however, was reported in some species. In tomato, it is observed near centromeres and in sugar beet, *in situ* hybridization with dinucleotide microsatellite motif suggested that some microsatellites repeats were highly clustered; but even if some clustering of microsatellite loci is observed, it is not a serious limiting factor for the development of microsatellite markers (Nunome *et al*., 2003).

2.4.3 Random amplified polymorphic DNA (RAPD)

RAPD markers have been described by Wellsh and McClelland in 1990 as well as Williams *et al*. (1990) and Nunome *et al*. (2001) as a simple and easy method to detect polymorphisms based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Pérez *et al*., 1998b). RAPDs are DNA fragments amplified by the Polymerase Chain Reaction (PCR) using short (generally 10 bp) synthetic primers of random sequence (Williams *et al*., 1990; Hadrys *et al*., 1992) with 60-70 % G+C content and with no self-complementary ends (Pérez *et al*., 1998b). These oligonucleotides serve as both forward and reverse primer and usually are able to amplify fragments from 3-10 genomic sites and more, simultaneously (Williams *et al*., 1990; Hadrys *et al*., 1992; Pérez *et al*., 1998b). A single oligonucleotide of random sequence is added in each reaction (Pérez *et al*., 1998b). A DNA amplification product is generated for each genomic region in the opposite direction between the priming sites (Pérez *et al*., 1998b). Amplified fragments (within the 0.5-5 kb range) are separated by gel-electrophoresis and polymorphisms are detected as the presence or absence of bands (Williams *et al*., 1990; Hadrys *et al*., 1992; Pérez *et al*., 1998b) of particular size. These polymorphisms may be caused either by failure to prime a site in some individuals (Williams *et al*., 1990; Hadrys *et al*., 1992) because of nucleotide sequence difference or by insertions or deletions in the fragment flanked by two priming sites (Pérez *et al*., 1998b). This method has considerable advantages over other kinds of DNA fragment analysis because it is fast and not expensive, can be used with limited amounts of DNA, and is suitable for work on anonymous genomes (Hadrys *et al*., 1992). It can, as opposed to other kinds of DNA fragment analysis like microsatellites, detect polymorphisms in any kind of sequence (Pérez *et al*., 1998b), but is very sensitive to small changes in reaction conditions, such as the type of polymerase, template DNA concentrations, magnesium concentration and temperature

profiling characteristics of the thermal cycler, as explained by Williams *et al*. (1990), Arnold *et al*. (1991), Carlson *et al*. (1991), Ellsworth *et al*. (1993) and Schweder *et al*. (1995) in Pérez *et al*. (1998b). Also bands of mixed intensity may arise with each primer (Pérez *et al*., 1998b), as well as artefactual ones from heteroduplex formation between amplified products as described by Ayliffe *et al*. (1994) or from other secondary artefacts stated by Hadrys *et al*. (1992) and Riedy *et al*. (1992), as cited in Pérez *et al*. (1998b). Another limitation of this technique is the nonspecificity of primers leading to low reproducibility of some of the amplified DNA fragments (Pérez *et al*., 1998b). Some authors, as in Pérez *et al*. (1998b), state that when amplifications are replicated, most of the bands are repeatable (Williams *et al*., 1990, Arnold *et al*., 1991); others report a low percentage of reproducible bands (Haig *et al*., 1994). Assuming exact priming, the expected number of bands for a given primer can be calculated as a function of its length and the size of target DNA (Clark and Lanigan, 1993), theory that fits well the number of amplified fragments in higher eukaryotes; in lower eukaryotes or prokaryotes (Larson *et al*., 1996; Ronimus *et al*., 1997), however, 10-mer primers often produce multiple bands where no bands are expected. The latest is explained by either misprinting or nonrandomness of genome sequences (Clark and Lanigan, 1993). RAPD technology has been used to estimate genetic diversity in a wide number of species, however, as read in Nunome *et al*. (2001), it is difficult to transfer the data from dominant RAPD markers to other segregating populations, which can be overcome by converting a marker framework covering the entire map into SCAR (Paran and Michelmore, 1993), CAPS (Konieczny and Ausubel, 1993) and RFLP (Nunome *et al*., 2001).

2.5 Conventional and unconventional breeding methods applied to eggplant

Eggplant is an autogamous diploid with 12 chromosomes (2n= 24), and its nuclear genome contains 1100 Mb of DNA. Despite eggplant's similarities to the other major solanaceous crops, there is rather little molecular genetic information for the species as compared to tomato, potato and pepper (Doganlar *et al*, 2002). Brinjal has a wide variability (Lal, 1991) in its morphological characters (colour, shape, size), physiological attributes and biochemical features; and exhibits partial resistances to most its pests and pathogens, but often at rather low levels, therefore, the introgression, through conventional breeding and biotechnological approaches (Table 2-8), of total or high resistance to diseases into the cultivated eggplant varieties is greatly needed (Collonnier *et al*., 2001), moreover, as far as abiotic stress is concerned, some traits of resistance against frost damage have been found (Baksh and Iqbal, 1979).

- Ploidy level of eggplant

The great majority of eggplant commercial cultivars belong to the Group Common Eggplant (*Solanum melongena*) and have diploid chromosome number (2n= 24). That chromosome number may be affected when biotechnological methods, e.g. protoplast culture or somatic hybridization are used to overcome sexual barriers between eggplant and it wild relatives (Collonnier *et al*., 2001; Kashyap *et al*., 2003). Somatic hybridization facilitates the transfer of useful traits into the cultivated eggplant from wild relatives, like disease and pest resistance, as well as quality and shelf life of fruits (Kashyap *et al*., 2003). However, when breeding of common eggplant is attempted through somatic hybridization with its wild relatives, one of the major limitations is generally represented by the tetraploid level of the somatic hybrids obtained. The reduction of the ploidy level to the diploid status is necessary to facilitate the backcross with the cultivated diploid eggplant in order to introgress useful traits (e.g. resistance to *Fusarium oxysporum* f.sp. *melongenae* and *Ralstonia solanacearum*) into the eggplant gene pool. The decrease of the ploidy level causes a dramatic reduction of the fertility of dihaploids, which is negative for their practical exploitation in breeding programmes by sexual crosses. Fortunately, the protoplast "backfusion" applied to dihaploids with eggplant opens up new possibilities to overcome sexual barriers between species (Rotino *et al*., 1997; Rotino *et al*., 2005).

Table 2-8 Breeding techniques applied to eggplant (Arpaia *et al*., 1997c; Ano *et al*., 1991a; Collonnier *et al*., 2001b; Acciarri *et al*., 2002d)

Technique		Objective	Advantage	Disadvantage
Conventional breeding	Sexual Hybridisation (a; b)	-improves agronomical traits through intraspecific crosses	introduction of useful characteristics from wild relatives into cultivated species	sometimes limited by sexual barriers
Biotechnological methods	Plant Regeneration and Somaclonal Variation (b)	-obtain available plant material in short time maintaining desirable features	positive response to *in vitro* culture; ability to regenerate *in vitro* via somatic embryogenesis and shoot regeneration	selection of somaclonal variation
	Haploidisation through *in vitro* androgenesis, [cultured anthers or isolated microspores] (b)	-obtains fixed lines from heterozygous material, -produces commercial F_1 hybrids	plant material available in short time for commercial production	androgenesis is genotype dependent
	Genetic Transformation via *Agrobacterium thumefaciens* (b; c)	-complements and supplements sexual hybridisation to improve existing varieties, -creates totally new germplasm, by inserting genes encoding for useful agronomic traits	*Bt* endotoxin and parthenocarpy genes have successfully been introduced so far	the introduction of new genes encoding resistance to biotic and abiotic stresses not done yet
	Genetic engineered parthenocarpy [DefH9-iaa M gene] (d)	-improves fruit productivity -increases marketable yield -obtains seedless fruits with higher quality	parthenocarpic eggplants have in average 33 % higher yield under greenhouse and field cultivation and reduce production costs	plants do not produce seeds for further breeding

2.6 Growing conditions of eggplant in the geographical centres of origin

It is widely believed that the wild ancestors of *Solanum melongena*, L. originated in the hilly equatorial regions of eastern Africa, while the advanced cultivars of eggplant developed in tropical Asia, more specifically in India, South East Asia and South China (Lester and Hasan, 1991). Africa is a vast continent, and it experiences a wide variety of climate regimes. The location, size and shape of the African continent play key roles in determining climate. Moderate to heavy precipitation associated with the Inter-Tropical Convergence Zone (ITCZ) characterizes equatorial and tropical areas. Because the movement of the ITCZ follows the position of maximum surface heating associated with meridional displacement of the overhead position of the sun, near-equatorial regions experience two rain seasons, whereas regions further poleward experience one distinct rainfall season. The mean climate of Africa is further modified by the presence of large contrasts in topography (Semazzi and Sun, 1995) and the existence of large lakes in some parts of the continent (IPCC, 2007). Tropical Asia is influenced predominantly by the monsoons. The region is physiographically diverse and ecologically rich in natural and crop-related biodiversity (IPCC, 1998). In tropical Asia, climate uniformity is differentiated by three factors: latitude, relief and continentality. The entire tropical Asia region stretches over 38° in latitude, so the differences resulting from this factor are pronounced. In spite of some differences, the climates of countries have one factor in common: The Asian monsoon modulates them all to a large extent. Several countries in this region have reported increasing surface temperature trends in recent decades. In Vietnam in South East Asia, annual mean surface temperature has increased over the period 1895-1996, with mean warming estimated at 0.32°C over the past 3 decades. The warming trend over India has been reported to be about 0.57°C per 100 years (Rupakumar *et al*., 1994). In tropical Asia, hills and mountain ranges cause striking spatial variations in rainfall. Approximately 70% of the total annual rainfall over the Indian subcontinent is confined to the southwest monsoon season (June-September). Also, the number of rainy days during the monsoon along east coastal stations has declined in the past decade. A long-term decreasing trend in rainfall in Thailand is reported (OEPP, 1996). From the facts exposed above, it is understandable why eggplant has been so much influenced by the geographical and meteorological conditions in its centres of origin. Along decades, all species had to adapt to climate changes in order to survive and strive, bringing as a consequence an increment in biodiversity that is a natural response to evolution and very useful for exploitation of the better adapted eggplant species, as well as the introgression of desired agronomical traits into the commercial genotypes.

2.7 Climatic requirement and fertilization in open cultivation system

Eggplant may be seeded directly in the field, but this is not usually recommended for it has several possible disadvantages (Granberry, 1990). Often appropriate weed control is more difficult with direct seeded than with transplanted eggplant. Direct seeding requires especially well made seedbeds and may require specialized planting equipment to adequately control depth of planting and in-row spacing. In addition, the field must be fairly level to prevent seeds from being washed away or covered too deeply with water-transported soil. Mostly, eggplant is transplanted in the field to provide the best opportunity for growth and fruit development under optimum temperatures. The advantages (Granberry, 1990), are that it allows a more efficient land use, and provides the best means of establishing a uniform and complete stand. It is advisable the hardening of eggplant seedlings before transplanting in the field, technique that slows plant growth prior to field setting so the plant can withstand unfavourable conditions in the field more successfully. For maximum production, transplants should not have fruits, flowers or flower buds before transplanting (Granberry, 1990; Lindgren, 1996).

2.7.1 Light and temperatures

The eggplant is a warm season crop (Prinz, 1989; Paksoy and Akilli, 1994; Rindels, 1997; Chen *et al*., 2001) that requires warm temperatures (21-22 ºC to 29-30 ºC) for optimal development and is very tender to frost (Lawande and Chavan, 1998; Chen *et al*., 2001). It can even be injured by periods of cold temperature above freezing, and is more sensitive to low temperatures than either tomatoes or peppers (Lindgren, 1996). The growth is negatively affected by temperatures under 16 ºC (Prinz, 1989; Lawande and Chavan, 1998). Cool temperatures (<21 ºC) can reduce fruit set and high (>29 ºC) ones as well as high humidity levels also reduce yields (Chen *et al*., 2001). For early production of eggplant in open field, sites with a southern to south-western light exposure are convenient (Granberry, 1990; Rindels, 1997); it grows best in full sun (Rindels, 1997) and as far as is known it is day length neutral (Prinz, 1989; Sutarno *et al*., 1993). A long growing season of about 120 days is required (Chen *et al*., 2001).

2.7.2 Soil and water consumption

Well-drained, sandy loam soils are ideal for the growth and development of an extensive root system of eggplant (Chen *et al*., 2001) with a pH from 5.5-6.0 to 6.5-7.2 (Prinz, 1989; Granberry, 1990; Rindels, 1997; Lawande and Chavan, 1998).

Poorly drained soils usually result in reduced functional root area due to the build-up of root-rotting pathogens, poor plant growth and low yields. Production in open field requires crop rotation to avoid the presence of pests and diseases (Chen *et al*., 2001; Chen *et al*., 2002). Eggplant is intolerant of poorly drained soil, so it is usually helpful, especially on heavier soils or in low areas to transplant eggplant on raised beds (Granberry, 1990; Lawande and Chavan, 1998). Even though this crop is tolerant of drought and excessive rainfall, generally, fruit set and production decrease under adverse conditions (Saito and Ito, 1973; Wang *et al*., 1980; Sun *et al*., 1990; Sutarno *et al*., 1993; Abney and Russo, 1997; Chen *et al*., 2001). Eggplant is a medium-rooted crop (Chen *et al*., 2001; fftc, 2005) with a root zone depth of 90 cm in well-drained soil. Irrigation, at least 45 cm deep, is essential during the growing season, being its most critical period during the time of flowering and fruit set. A lack of water during this period could lead to the development of blossom-end rot and malformed fruit as well as reduction of fruit size and yield (Chen *et al*., 2001). Generally, irrigating the field every 3rd or 4th day in summer and every 10th to 14th day in winter is considered adequate to maintain proper soil moisture. Eggplant is found to respond well to drip irrigation, which not only increases yield but also decreases weed population (Lawande and Chavan, 1998). Mulching with dried plant materials (Chen *et al*., 2001) or with thin black polyethylene sheets reduces moisture loss and weed problems (Sutarno *et al*., 1993).

2.7.3 Nutrients and fertilization

The solanaceous group of vegetables including tomato, eggplant, chili and bell peppers, generally, takes up large amounts of nutrients. That depends on the quantity of fruit and dry matter they produce, which in turn is influenced by a number of genetic and environmental variables. Fruit and fruiting parts in this group of vegetables contain 45 to 60% of total N, 50 to 60% of total P, and 55 to 70% of total K absorbed by the plants (fftc, 2005). The major proportion of the nutrients in fruit is absorbed from the time of flowering (Chen *et al*., 2001; Chen *et al*., 2002; fftc, 2005). The period of greatest nutrient requirements for N, P and K is from about ten days after flowering to just before the fruit begins to ripen. There is diurnal variation in nutrient absorption. A higher proportion of P tends to be absorbed during the night than N or K (fftc, 2005). Eggplant is very effective in making use of plant nutrients already available in the soil, even though this crop use only a small proportion of N from organic sources (fftc, 2005). Before planting eggplant, the field is manured with compost (10 t/ha) or chicken manure (3 t/ha) or at planting a complete mineral fertilizer solution is applied (Chen *et al*., 2001; Chen *et al*., 2002).

Eggplants are heavy feeders (Rindels, 1997), therefore, additional fertilizer dressings of nitrogen, and in some cases also potassium are beneficial to the crop 4 to 6 weeks after transplanting. There may be need of additional fertilization, which is dependent on local conditions (Sutarno *et al.*, 1993; Lawande and Chavan, 1998; Chen *et al.*, 2001). In the sandy loam soils at AVRDC (Chen *et al.*, 2001; Chen *et al.*, 2002), typical fertilizer rates are 170 kg/ha of N, 70 kg/ha of P_2O_5, and 180 kg/ha of K_2O. Recommendations for the distribution of fertilizer during the growing season are shown in (Table 2-9). Using nitrogen (N) as an example, 30% of the total N should be applied before transplanting (basal), 15% applied in each side dressing (3 and 6 weeks after transplanting), and 40% applied during the harvest, typically in 4 applications, each spaced 2 weeks apart.

Table 2-9 Distribution of fertilizer for eggplant field cultivation (Chen *et al.*, 2001)

Nutrient	Basal	3 wks	6 wks	During harvest	Total
N	30%	15%	15%	40%	100%
P	50%	0	50%	0	100%
K	30%	15%	15%	40%	100%

The quantity of nutrients to be applied depends on the yield potential of the cultivar, the level of available nutrients in the soil, and growing conditions (Chen *et al.*, 2001; Chen *et al.*, 2002; fftc, 2005). Because in this crop vegetative and reproductive stages overlap, and because the plants need nutrients even up to fruit ripening, application methods such as fertigation, split application of fertilizers, use of slow release N fertilizers, and integrated use of fertilizers and organic sources of nutrients have proved very effective in increasing nutrient use efficiency and crop productivity, and reducing nutrient losses (fftc, 2005).

2.8 Growing conditions for eggplant in greenhouse cultivation system

Eggplant requires a tropical to subtropical ecosystem to strive. It is necessary warm temperatures, fairly humid air (40-60%), well-drained and humid soil (not saturated) that enhances root development, and a relative high irradiation (Chen *et al.*, 2001; AVRDC, 2001). Thus, protected cultivation gives the possibility of growing tropical crops, like eggplants, in temperate regions. The breakthrough of protected cultivation is that the environment can be modified in favour of plant growth and productivity (Kavetskiy *et al.*, 2003; Bougoul *et al.*, 2005). Among the many advantages of greenhouse cultivation systems are the ability to improve environmental conditions for the crop, the possibility to extend the production season, the chance to increase crop range, independently of local climate conditions, and it solves the serious pathologic problems caused by soil borne contamination (Kavetskiy *et al.*, 2003; Bougoul *et al.*, 2005).

Therefore, the climate conditions for eggplant can be easily simulated under protected cultivation system in temperate areas as they occur in the regions where this crop originates. The biggest constraint of protected cultivation in mild-winter climates, nevertheless, is the irradiation, which is a decisive climatic factor with strong influence on eggplant´s physiology. Fortunately, the solution to this potential problem lies in the genetic variability of eggplant, through the selection of early flowering genotypes that do not command the extra use of artificial light and as a result, the production of this crop do not necessarily results on more expenses by electricity consumption.

2.8.1 Types of Greenhouses

Greenhouses are classified (NSWDPI, 2005) according to:

-shape (gable, flat arch, raised dome, sawtooth, skillion, tunnel)

-structure (multi-span, crop top structures, shade and screen houses)

-technology (low, medium and high technology greenhouses)

Multi-span greenhouses are robust in design, have a surface area smaller than a number of single span greenhouses of equivalent production capacity, which results in less heat loss and significant energy savings.

A crop top is a structure with a roof but which does not have walls. The roof covering may be a greenhouse covering material such as plastic or glass, or shade cloth or insect screening. These structures provide some modification of the growing environment such as protection of the crop from rain or reduction of light levels.

Shade houses are structures which are covered in woven or otherwise constructed materials to allow sunlight, moisture and air to pass through the gaps. The covering material is used to provide a particular environmental modification, such as reduced light or protection from severe weather conditions. The height of the structure will vary according to the type of crop being produced and may be as high as 8 metres.

Screen houses are covered in insect screening material instead of plastic or glass. They provide environmental modification and protection from severe weather conditions as well as exclusion of pests. Both shade and screen houses are mostly used in warmer regions.

Low technology. These greenhouses are less than 3 metres in total height, common types are tunnel houses, or "igloos" that do not have vertical walls and have poor ventilation. This type of structure is relatively inexpensive and easy to erect with little or no automation at all. While this sort of structure provides basic advantages over field production, crop potential is still limited by the growing environment and crop management is relatively difficult. Low level greenhouses generally result in a suboptimal growing environment which restricts yields

and does little to reduce the incidence of pests and diseases. Pest and disease control, as a result, is normally structured around a chemical spray program. They have significant production and environmental limitations, but they offer a cost effective entry to the industry.

Medium technology. These greenhouses are typically characterised by vertical walls more than 2 m but less than 4 metres tall and a total height usually less than 5.5 metres. They may have roof or side wall ventilation or both, usually clad with either single or double skin plastic film or glass and use varying degrees of automation. Medium level greenhouses offer a compromise between cost and productivity and represent a reasonable economic and environmental basis for the industry. Production in medium level greenhouses can be more efficient than field production.

High technology. These greenhouses have a wall height of at least 4 metres, with the roof peak being up to 8 metres above ground level. These structures offer superior crop and environmental performance. High technology structures will have roof ventilation and may also have side wall vents. Cladding may be plastic film (single or double), polycarbonate sheeting or glass. Environmental controls are almost always automated. These structures offer enormous opportunities for economic and environmental sustainability. Use of pesticides can be significantly reduced. High technology structures provide a generally impressive sight and, internationally, are increasingly being involved in agribusiness opportunities. Although these greenhouses are capital intensive, they offer a highly productive, environmentally sustainable opportunity for an advanced fresh produce industry.

2.8.2 Cultivation Systems in Greenhouses: Soil and soilless

The methods to produce vegetables in a greenhouse include (Boyhan *et al*., 2000; Kavetskiy *et al*., 2003; EPA, 2007):

- planting directly in the soil within the greenhouse
- substrate culture that includes: use of substrate layers and soilless system or hydroponic

- Soil System

Planting directly into the soil requires the least amount of initial labor, but there is a risk for soil born contamination and weed problems. These problems can increase over time with successive cropping, particularly if the same crop is grown repeatedly. Fumigating the soil may be the solution, although care must be taken when using a chemical fumigant in an enclosed structure. Soil solarisation, in which the greenhouse structure itself acts as the solarising unit, may control some problems with the soil system, specially, if the soil is turned and moistened during the hot summer months. Soil fertility should be determined and managed with soil testing. As with any field soil, the soil pH should be adjusted to 6.0 to 6.5.

Soluble salts accumulation can be particularly severe with the soil system. To minimize this problem, the house can be uncovered when not in production to allow rainfall to leach the soil. Plants should be mulched to control weeds, retain soil moisture and prevent soil compaction. Plants can be set in plastic mulch, or organic mulches can be used.

- Substrate Culture (Container System)

Planting in a typical potting media (combined with soil or not) can also be successful. Many different manufactured media are available, most of which are peat based and include various blends of peat with perlite, vermiculite, sand or ground pine bark. Fertilizer sources can be either soluble or slow-release formulations. Unfortunately, the amount of fertilizer incorporated in these media is not sufficient to carry the crop to completion and these media can harbor various pathogens that may be introduced to the greenhouse over time, because the high organic matter present is an ideal substrate to support the growth of microorganisms. Usually, large quantities of media are costly. Substrate culture is applied when the soil conditions do not satisfy crop requirements, then either **substrate layers** (combination of soil and substrate) or **hydroponic** (soilless system) is used.

Substrate layer can be combined with drainage and heating system in the rhizosphere, and with tubes for soil/substrate steaming. Materials like wood (high stability), peat (improves water capacity), manure or straw (favour biological activity) are advisable to use for such system. For best results the following factors should be considered: substrate shrinkage, soil disinfection, extra supply of CO_2, use of trickle irrigation and heating tubes in the root zone.

Hydroponic systems increase the efficiency of water use because all the nutrients required by the plant are supplied in the irrigation water, and offer greater opportunity to use non-chemical pest and disease management strategies. According to the use or not of substrate, hydroponic systems are classified as:

-Substrate culture (3 to 15 L of substrate per plant) in containers, bags, slabs or channels. Preferably, rockwool slabs are used combined with different types of trickle irrigation. The nutrient solution can be recycled (closed hydroponic system), or discharged after use (open hydroponic system).

-Water culture (no substrate at all except the one for propagation or gravel culture to stabilise the root zone) in which the plants are hanging in channels in recirculating nutrient solution. Most known types are: the Nutrient Film (NFT) and the Deep Flow (DFT) Techniques.

-Aeroponics (no substrate at all) where the plants are holding on plastic foam sheets and the roots receive the nutrient solution by very fine nozzles (2-4 minutes for 20-30 seconds) that form a foggy environment in a closed space under the foam sheets. In this system the plants

are kept in beds (1-1.20 m wide, ca. 30 deep, ca. 80 cm high) isolated with plastic foil.

The various methods of hydroponic production have become standard for producing greenhouse vegetables. These methods are environmentally friendly and very clean, with no organic material present, and they give the grower complete control over the crops' nutritional needs, which results in maximising growth and fruit production. Hydroponics bring both nutrients as well as fresh oxygen to the root zone and take away "off-gases," the waste by-product of the root zone, making it a highly efficient and cost-effective technology. Besides, because nutrients are readily available in hydroponic systems, plants have smaller, more efficient root systems and can spend more energy growing the more valuable above ground stems, foliage, and fruit. Furthermore, growers can space plants closer together, thus producing more agricultural products per a given area, while avoiding competition for scarce nutrients in the root zone. Plants can be grown in various media in these systems; all are essentially nutrient free, for example: rockwool, turf stone, clay granules, sawdust, flexible polyurethane foaming blocks, composed hardwood bark, or peat. The advantages of hydroponic or soilless cultures on artificial substrates are:

- standardisation of growing systems
- independence of growing place
- better regulation of nutrient and water supply
- absence of competing weeds and soilborne pests and toxic residues;
- water conservation (with recycling systems, hydroponic systems use one tenth the amount of water used in irrigated agriculture);
- growing conditions that can be altered quickly to suit specific crops, various growth stages, and environmental/climate conditions.

Hydroponic systems are mostly utilized in indoor greenhouses in non-tropical climates, allowing the grower to have control over climate conditions. Even though, they may be used in the open for cultivation of nursery plants and in subtropical or tropical regions for vegetable production.

The disadvantage of hydroponics is that it requires calculating and measuring exact amounts of fertilizers, which means handling several different fertilizer compounds. Mistakes in calculations or quantities used are more likely to show up as deficiencies or toxicities in the crop. It is particularly important for hydroponics that regardless of the production system most will require some type of automation to supply water and high initial capital cost. Besides, fungal diseases may spread rapidly when closed substrate culture or NFT is used.

2.8.3 Greenhouse production systems

Greenhouse production of vegetables, in general, is based on either soil or soilless culture. In soil culture, vegetables are produced in the existing soil or in a loamy soil brought inside the greenhouse. Soilless culture ensures the production of plants in a soilless medium. It can be done via hydroponics or with an artificial substrate (Resh, 1991; Greer and Diver, 2000). In 1974, 70 percent of U.S. production was based on soil culture and 30 percent on soilless culture. By 1988, a significant shift to soilless systems had occurred, with soil systems making up only 40 percent of the acreage (Greer and Diver, 2000). The majority of large producers in Canada raise tomatoes, cucumbers and peppers in rock wool, and lettuce is usually raised with a nutrient film technique (NFT) system (Greer and Diver, 2000). The reasons soilless culture became so popular are: a) the elimination of fumigation and steam treatments for soil-borne diseases; b) ease of installation; c) technical assistance available for soilless systems; and d) fertility control through commercial soluble fertilizers (Resh, 1991; Greer and Diver, 2000). Besides, because plants are not grown in soil there is no need to cultivate weeds, neither to rotate crops; and above all, fruit quality is higher (Resh, 1991). In the case of eggplants, particularly, the seeds are sown in early summer. The seedlings are kept from 60 to 80 days in a nursery. Eggplant demands high light intensity and warm temperatures. Therefore, the protected cultivation area is oriented from south to north and artificial heating is used in winter in temperate climates. Optimal temperature in the rooting zone is around 20 ˚C. Eggplant cultivars for protected cultivation are usually trained in a V shape with up to 3 main shoots per plant to facilitate the penetration of light deep in the canopy and the plants may be sprayed with plant growth regulators to ensure fruit set (Tachibana, 1994).

2.9 Young plant production

2.9.1 Generative propagation

- Seed production

The commercial propagation of eggplant is done by seeds (Sutarno *et al*., 1993; Chen *et al*., 2001). To ensure a profitable yield the starting material must be of first quality. Hence, several studies focus on the factors affecting the quality of eggplant seed production. Petrov *et al*. (1981) found that seeds obtained from fruits of the first and second level are of highest 1000-seed weight, germination energy and germinability. The stage of maturity influences the quality of seeds and substantiates earliness. Highest quality seeds are obtainable from eggplant fruits harvested in full botanical maturity. Usually, aubergines are best consumed

when the fruits are not ripe. Therefore, a considerable part of the harvest should be kept for the obtention of seeds (Sutarno *et al*., 1993; Chen *et al*., 2001). Alternatively, seeds or seedlings may be bought from specialised companies (Granberry, 1990). The post-harvest storing of fruits in the course of three to five days has been found to influence most favourably the 1000-seed weight, the germination energy and the germination capacity of the seeds. It is also shown to improve the productivity of plants originating from such seeds (Chen *et al*., 2001).

- Germination and young plant production

Soaking the seeds in water for 24 hours hastens germination. The practice is to sow in a shaded seed-bed (Sutarno *et al*., 1993; Chen *et al*., 2001) in a nursery. The seedlings appear after 8-10 days then they can be pricked out in small pots 2-3 weeks later. Transplantation to the field of the seedlings takes places 3 (Sutarno *et al*., 1993) to 5 (Chen *et al*., 2001) weeks after, on raised beds or ridges (Sutarno *et al*., 1993). Seedling production can take place under greenhouse conditions in cells, plug trays or containers because they allow field planting without disturbing the root system. The containers are filled with a sowing medium such as peat moss, commercial potting soil, or a potting mix prepared from a combination of soil, compost, rice hulls, vermiculite, peat moss and sand. It is recommended to sterilize the soil mixture by autoclaving or baking at 150 °C for 2 hours and to fertilize the seeds weekly after two weeks, preferably with a water-soluble fertilizer solution. Plug seedlings will be ready to set in the field four to five weeks after sowing (Chen *et al*., 2001; AVRDC, 2001). Seeds may also be sown (rows 6 cm apart and 0.5 cm deep) in seedling beds (15 cm high and 0.8 m wide). The seedbeds should be fertile and well drained. The bed area can be incorporated with fertilizers at 40 g/m^2 ammonium sulfate, 50 g/m^2 superphosphate, 30 g/m^2 potassium chloride, and 2 kg/m^2 of compost. Usually, a thin layer of compost is applied on the bed before mulching with rice straw. Then, cover them with a mesh screen net. Thin seedlings at the first true leaf stage will be ready for transplanting in five to six weeks after sowing (Chen *et al*., 2001; AVRDC, 2001).

2.9.2 Vegetative propagation

- Shoots and Grafting

Propagation of eggplants by rooting of healthy shoots is possible (Sutarno *et al*., 1993; Sutarno *et al*., 1993). The main objective of grafting is to avoid the soil-borne disease bacterial wilt in *Solanaceae*. Inter-generic grafting is used in the production of many fruit-bearing vegetables, while inter-specific grafting is generally applied to eggplant (*Solanum melongena* L.). Scarlet eggplant (*S. integrifolium* Poir.) and *S. torvum* Swartz are popular

rootstock for eggplant production (fftc, 2005). Cleft and tube grafting are the methods used for grafting eggplants. Other possibilities are the tongue approach and cut methods for grafting other fruit bearing vegetables (fftc, 2005). Grafting should be carried out in a shady place sheltered from the wind, to avoid wilting of the grafted plants. The healing and acclimatization are very important for grafted plants to survive. During acclimatization, it is recommended to keep light levels at about 3 to 5 Klx (fftc, 2005). Before grafting, it is important to expose the scion and rootstock to sunshine for two to three days, to withhold water from the plants to avoid spindly growth, and to make sure that the scions and rootstock have stems of a similar diameter (Oda *et al*., 1994). Once grafting is performed, it is important to increase the chances for vascular bundles of the scion and rootstock to come into contact (Oda *et al*., 1994), by maximizing the area of the cut surfaces that are spliced together, and by pressing the spliced cut surfaces together. The cut surfaces should not be allowed to dry out (fftc, 2005). After grafting, keeping the grafted plants at about 30°C and with more than 95% relative humidity for three days of healing promotes the survival ratio. Gradually, the relative humidity is then lowered and the light intensity increased. During healing and acclimatization, it is important to keep a constant air temperature around the grafted plants, in order to maintain high humidity. If wilting is observed, foliar spraying of grafted plants with water is effective in helping them survive (fftc, 2005).

- *In vitro* propagation of eggplant

Agricultural biotechnology plays a special role in breeding programmes and cloning of elite species. Among its most practically applied technique is the tissue culture including plant regeneration methods like organogenesis and somatic embryogenesis. Also, germplasm conservation, massive propagation, plant breeding by *in vitro* mutagenesis and selection and genetic engineering to obtain transgenic organisms are frequently used biotechnological methods (Pérez *et al*., 1998a). A biotechnological method like the tissue culture technique is among the most popular approaches for the study and multiplication of agricultural important crops (Jiménez, 1998). Tissue culture allows under sterile conditions the culture of organs, tissues, cells and protoplasts using artificial nutritive media. *In vitro* propagation can be used for multiplication of genetically identical organisms or clones and it is an integral part of genetic transformation procedures (Gubis *et al*., 2004). It is applicable in theoretical studies about physiology and biochemistry of plants (Nash and Davies, 1972; Komamine *et al*., 1982); for obtaining pathogen-free plants (Morel and Martin, 1955), in the massive propagation of plants (Vasi, 1994; Kitto, 1997), in plant germplasm conservation (Withers, 1985), in the production of secondary metabolites (Misawa, 1994), in plant genetic breeding

by *in vitro* mutagenesis and selection (Pérez *et al*., 1998a), as well as in genetic engineering (Herrera-Estrella *et al*., 1983). Eggplant responds well to different tissue culture techniques, thus facilitating the breeding of this economically important crop through the use of biotechnology. Plant regeneration of this vegetable is possible through *in vitro* organogenesis, either direct or indirect; or somatic embryogenesis (Collonnier *et al*., 2001; Kashyap *et al*., 2003), and can be efficiently regenerated from cultured roots as well (Franklin *et al*., 2004). Moreover, *in vitro* genetic variability resulting from anther culture of this crop can be used for practical breeding purposes (Rotino *et al*., 1992). It is also possible to use tissue or cell culture to increase genetic variability (Boxus, 1998), because undifferentiated cells obtained from callus, cells or protoplasts culture are produced and then submitted to a selective pressure to fix the somaclonal variants. Micropropagation speeds up the propagation process for several vegetatively propagated crops (Heffer, 2001), and its main advantage is the propagation in a short period of time of high quality and healthy planting material, maintaining genetic stability as well. The *in vitro* plant propagation is among the most advanced and modern techniques in plant nursery and horticulture. To date these techniques are applied at both industrial and research levels, e.g. to set pathogen-free clones, micropropagation, embryo rescue, keeping male sterile and polyploid stocks, induction of somaclones, protoplast fusion and genetic transformation (Collonnier *et al*., 2001; Cammareri *et al*., 2004).

2.10 Cultivation systems for eggplant

2.10.1 Cultural practices common to eggplant production

There are several agronomic practices that favour the growth of eggplant, independently of the production system in use (Table 2-10) summarizes the most common cultural practices applied to this crop.

Table 2-10 Cultural practices applied to eggplant

Cultural Practice	Objective	Cite
Transplanting	Allows easier weed control, more efficient land use and earlier Spring harvest dates, no need for especial well made seedbeds and specialized planting equipment that controls depth of planting and in raw spacing, usually replanting is not required and prevents washing away of seeds or covered too deeply with water transported soil	Granberry, 1990 Chen *et al*., 2001
Hardening off	It slows down plant growth so the plant can withstand unfavourable conditions more successfully and reduces transplanting shock	Granberry, 1990 Chen *et al*., 2001
Mulching	It reduces weeds, prevents soil compaction and conserves soil moisture. Plastic much is outstanding for preventing weeds, while organic mulch cools soil temperatures, so usually these mulches are often used in combination during the hot growing season	Chen *et al*., 2001 Chen *et al*., 2002
Fertilization	Adequate combination of manures and fertilizers results on successful crop production. Eggplant is a long duration crop and requires large amount of fertilizers that are distributed during the growing season. Organic fertilizers improve soil structure and conditions; and are applied before transplanting	Chen *et al*., 2001 Chen *et al*., 2002
Irrigation	Beneficial to transplanted seedling until the plants are well established during deficient water conditions, maintains enough available moisture for active plant growth, is most critical during flowering and fruit set and enhances fruit size and yield	Granberry, 1990 Chen *et al*., 2001
Weed control	Eggplant is slow to become established and can not compete with weeds. Besides, weeds harbour damaging insects and diseases	Chen *et al*., 2001
Staking and Pruning	Supports the plant from fruit load, allows more air circulation and lighting within the canopy and enhances production of bright-coloured, high quality fruit	Chen *et al*., 2001 Chen *et al*., 2002
Pollination	Especially recommended for crossing of genotypes. Pollinators are absolutely necessary for fruit set and seed formation, therefore, insect pollination is required for good yields	Granberry, 1990 Chen *et al*., 2001 Pessarakli & Dris, 2004

2.10.2 Density and branching of plants

Depends on number of branches, crop cycle, development of the variety, cultivation system and type of greenhouse. Generally, the most used approaches are listed in Table 2-11 (Mangione and Sánchez, 1999; infoagro.com, 2003).

Table 2-11 Density of eggplant according to number of branches

No. of branches	Spacing	
	between plants (m)	between raws (m)
4 or more	0.5	2
4	0.75	1.50
3-4	0.5	1.75
3	0.5	1.50
2	0.5	1

Often, the density of eggplant considers the amount of individuals in the production area for this crop (infoagro.com, 2003), either in open field or protected cultivation system (Table 2-12).

Table 2-12 Density of eggplant according to cultivation system

Cultivation system	Spacing						
	Single raw			Double raw			
	between plants (m)	between raws (m)	crop density (p/ha)	between plants (m)	between raws (m)	every 2nd raw (m)	crop density (p/ha)
Greenhouse	0.60	1.50	11 000	0.50	0.90	1.40	15 000
				0.60	0.50	1.00	22 000
Open field or tunnel	-	-	-	0.50	0.90	1.60	16 000

2.11 Open issues and problem statement

Eggplant's yield partly depends on environmental factors (Saito and Ito, 1973; Wang *et al*., 1980; Sun *et al*., 1990; Abney and Russo, 1997). Fruit set is sensitive to high relative humidity, high night air temperature and low light levels (Saito and Ito, 1973; Wang *et al*., 1980; Sun *et al*., 1990; Abney and Russo, 1997). Besides, this crop is susceptible to numerous diseases and parasites (Sihachakr *et al*., 1993), for which shows insufficient resistance (Daunay *et al*., 1991; Collonnier *et al*., 2001; Kashyap *et al*., 2003). It is known, nevertheless, that many of the solanaceous species related to eggplant are resistant (Daunay *et al*., 1991; Collonnier *et al*., 2001; Kashyap *et al*., 2003) to the biotic and abiotic stresses negatively affecting the development of commercial eggplant varieties. Primitive cultivars of *Solanum melongena* can still be found in Asian countries (Lawande and Chavan, 1998; Collonnier *et al*., 2001), but crossability between the cultivated eggplant and other *Solanum* species is very low (Daunay *et al*., 1991; Collonnier *et al*., 2001; Kashyap *et al*., 2003) and limited by sexual barriers (Franklin *et al*., (1995), Collonnier *et al*., 2001; Kashyap *et al*., 2003).

Biotechnological approaches and conventional breeding methods to improve agronomical traits of eggplant include: sexual and somatic hybridisation, haploidisation, genetic transformation, direct and indirect plant regeneration as well as somaclonal variation aiming at the introgression of total or high resistance to abiotic stress, pests and diseases, yield, fruit quality and shelf life improvement (Collonnier *et al*., 2001; Kashyap *et al*., 2003). Although, biotechnological approaches exceed traditional breeding by overcoming sexual barriers between species; and many hybrids have been obtained in this way, there are still two situations to look after: when crosses are successful, the fertility of the progeny is often too low for further use in breeding programmes, and even though the wild relatives have valuable characteristics, they must be used with caution because they may be very susceptible to *Colletotrichum gloeosporioides* and have high level of the steroid saponin responsible of the bitter taste found in *S. torvum* and *S. linneanum* (Collonnier *et al*., 2001; Kashyap *et al*., 2003). The wide utilization of hybrids has been detrimental to the genetic biodiversity of eggplant (Collonnier *et al*., 2001). An alternative to conventional breeding and biotechnological methods, could be the selection of new eggplant genotypes from varieties or landraces from one of the crop's geographical centres of origin. Therefore, screening of cultivars occurring in the centre of origin of this crop in South East Asia may result in the selection of new genotypes carrying desirable agronomical traits.

3 Aim of the thesis

3.1 General objective

Eggplant is a relatively easy to cultivate and high-yielding vegetable and it is considered a vegetable of tropical and subtropical regions that requires high light levels (Lawande and Chavan, 1998). Nevertheless, according to the problems described above, climate as well as lack of resistance to many of its pests and diseases, have a negative influence on the development and yield of this crop. The commercial production of eggplant under protected cultivation may help to circumvent adverse weather conditions. That is important in countries with temperate climates, where open field production of eggplant would be impossible due to low temperatures. The aim of this research consisted of contributing to increase the number and diversity of eggplant in temperate regions by selecting new stable genotypes for greenhouse production systems, which may be interesting for breeding programmes of the species in general, and of practical use for the commercial production of *S. melongena* in Western Europe, particularly in Germany.

3.2 Specific objectives

In order to achieve the goal of the research, experiments were design and carried out to:

-evaluate the yield (fruit load, fruit number) of the Asian eggplant genotypes in the greenhouse and compare it to that of typical „European" cultivars "RZ Adona" and "RZ Ritmo",

-compare both, the vegetative (plant height, leave number) and generative (days to first flower, flower intensity, fruit set percentage) development, of the Asian eggplant genotypes with that of the cultivar "RZ Adona", under controlled environmental conditions,

-establish the relationship between a morphological or physiological feature and the expected yield; e.g. beginning of the flowering period of the Asian eggplant genotypes,

-evaluate the response of 5 eggplant genotypes to micropropagation and *in vitro* manipulation, e.g. callogenesis, organogenesis, plant regeneration from calli,

-study the influence of PGR, e.g. NAA and 2,4 D, in the capacity of eggplant for callus and organ formation, and indirect plant regeneration *in vitro*,

-study the effect of both, organic and inorganic substrates, on the development of eggplant in the greenhouse.

-get insight on the genetic differences of eggplant genotypes from distinct geographical origin via RAPD-PCR, and

-achieve basic knowledge about the genetic variability of the different clones of the Asian eggplant genotypes involved in this research.

3.3 Hypothesis

The research was based upon following hypothesis:

There is a wide variety of eggplant genotypes in the species's geographical centres of origin. Therefore, the selection of new stable genotypes suitable for greenhouse cultivation will be achieved by exploiting the diversity of local varieties bred in South East Asia.

Cultivation of the new selected eggplant genotypes of Asian origin will be possible on substrate culture with drip irrigation under protected cultivation in temperate regions.

In vitro plant culture is a tool for mass propagation of eggplant. Thus, micropropagation and acclimatization of Asian eggplant genotypes shall be used to speed up the selection process.

Additionally, increasing the agrodiversity of eggplant in temperate regions is meaningful for local markets that will need to introduce "exotic" vegetables, and will be highly appreciated by Asian communities leaving there that still prefer their typical vegetables.

3.4 Research questions

1. How the Asian eggplant genotypes respond to tissue culture techniques?
2. Is eggplant's phenotypical diversity linked to a genetic marker?
3. Have organic or inorganic substrates for greenhouse cultivation an influence on the growth of the Asian eggplant genotypes?
4. How are the vegetative and generative developments of the Asian eggplant genotypes under greenhouse conditions?
5. Is there a relationship between the beginning of the flowering period of the Asian eggplant genotypes and the yield?
6. Is it possible to obtain stable eggplant genotypes for greenhouse cultivation system by screening eggplant landraces of South-Asian origin?
7. Would the new selected Asian eggplant genotypes strive for similar harvest like the high yield varieties "RZ Adona" and "RZ Ritmo", in a commercial-like, 'substrate culture' cultivation system in greenhouse?

4 Material and Methods

The investigation consisted of 5 experimental phases that include experiments designed to achieve the research's objectives by the germination and greenhouse cultivation, micropropagation of the plant material, the evaluation of morphological characteristics and generative growth of the eggplant genotypes, and the assessment of production potential, as well as genetic stability of selected eggplant genotypes of Asian origin in greenhouse cultivation system. Detailed information on each of the experiments is presented in the following subchapters.

4.1 Overview of the experimental programme

The experiments carried out for this research pursued five aims (Table 4-1):

- finding out suitable propagation method for selected genotypes
- registration of vegetative growth (number and size of leaves, plant height)
- monitoring of generative growth (number, prematureness and fertility of flowers)
- evaluation of the harvest (number, weight, size, colour and shape of fruits)
- adaptation of selected genotypes to different greenhouse growing conditions (temperature, light, humidity, substrates, irrigation systems and nutrition)

Experimental phase I

- Handling and evaluation of the plant material under greenhouse conditions

Seeds of the eggplant genotypes "RZ Adona", "1507", "Cao San", "Dok" and "VASI-5" were placed in the germination chamber on the 6.08.2001 during 14 days at 20°C (night temperature), 26°C first day and 26°C the remaining germination period both, day and night. After germination 30 seedlings (6 per genotype) were hardened until each plantlet had 4 grown leaves, and then transplanted after 5 weeks to the 8 L containers with the organic substrate B_{700} (Att. I). Another 30 seedlings (6 per genotype) were transplanted at the same age to the 8 L containers with the inorganic substrate perlite. In order to assess the genetic variability of the eggplant genotypes, a morphological evaluation of the plant material as well as both physiological and agronomical traits were investigated (Table 4-1).

- Handling of the plant material *in vitro* and 1st selection of eggplant clones

In parallel, another group of seeds of the above listed eggplant genotypes were given a number, and then germinated *in vitro*. Afterwards the germination percentage was evaluated and the respective seedlings were established and multiplied or cloned *in vitro* every 5 weeks during $4_{1/2}$ months. Following the second subculture a pre-selection of the numbered clones took place based upon their multiplication rate (Table 4-1).

A total of 7 plantlets were pre-selected belonging to clones 6 and 12 of "RZ Adona". The exact number of pre-selected plantlets per clone and genotype of the Asian group of plants is listed in Att. IV.

Experimental phase II

- Evaluation of eggplant clones in the greenhouse

For this experimental phase, plantlets of the above named eggplant genotypes were cloned and propagated *in vitro*, then acclimatized and transplanted in the greenhouse.

The pre-selected clones were placed in the acclimatization room in trays during 14 days at 24°C under lamps type OSRAM L 58/ 31-038, and then transplanted for further development in the greenhouse to 8 L containers filled with the organic substrate B_{700}.

The morphological and physiological evaluation of the plant material was done with the determination of the flowering beginning period and the investigation of agronomical traits, as well as the selection in the greenhouse of the clones for the last 2 experiments of this study. Seeds of the selected clones were extracted from mature fruits and conserved (Table 4-1).

- Handling of eggplant clones in the laboratory

In the plant tissue culture laboratory, the micropropagation for conservation of the plant material was continued. Besides, in the molecular biology laboratory, a preliminary genetic analysis was carried out to assess the genetic variability of the plant material via RAPD-PCR technique (Table 4-1).

Experimental phase III

- Evaluation of eggplant clones in the greenhouse

In this experimental phase the evaluation of the selected clones was done regarding the flowering beginning and the flower production period, the percentage of fruit set as well as the yield (Table 4-1).

- Handling of eggplant clones in the laboratory

The refreshing and conservation of the selected clones *in vitro* was undertaken (Table 4-1).

Experimental phase IV and V

- Evaluation of eggplant clones in the greenhouse

In the last 2 experiments the evaluation of the Asian clones continued focusing in the performance *in vivo* of those new eggplant types under greenhouse cultivation. The high yield variety "RZ Ritmo" was used as control replacing the cv. "RZ Adona" in these experimental phases, and the selected eggplant genotypes were tried in a commercial-like greenhouse cultivation system. Specifically in the last experiment, seeds and plantlets obtained *in vitro* of the selected clones were used for propagation of the eggplant genotypes, in order to evaluate their genetic stability (Table 4-1).

Table 4-1 Experimental complex

Experimental phase	Technique	Propagules	Parameter	Goal
I (Winter 2001-02)	Micropropagation (MP)	Seed& Sedlings	Germination frequency Multiplication factor	Micropropagation of eggplant genotypes to quickly obtain clones & pre-selection of clones
	Greenhouse Cultivation (GC)	Seed	Vegetative & Generative Growth	Assessing genetic variability of the Asian genotypes
II (Summer 2002)	MP	Plantlets of *in vitro* origin	Multiplication factor	Micropropagation of eggplant clones
	RAPD-PCR analysis	Fresh leaf tissue from *in vitro* seedlings	DNA Fingerprint	Getting insights into the genetic pattern of the clones
	GC	Plantlets of *in vitro* origin	Vegetative & Generative Growth	Evaluation of pre-selected clones regarding morphological, physiological & agronomical traits
III (Summer 2003)	MP	Plantlets of *in vitro* origin	Multiplication factor	Micropropagation & conservation of eggplant clones
	GC	Plantlets of *in vitro* origin	Vegetative & Generative Growth	Evaluation of the clones from Asian genotypes with early flowering feature & comparable harvest like “RZ Adona”
IV (Summer 2004)	GC	Plantlets of *in vitro* origin	Vegetative & Generative Growth	Evaluation of the clones from Asian genotypes with early flowering feature & comparable harvest like “RZ Ritmo”
V (Summer 2005)	GC	Seeds & Plantlets of *in vitro* origin	Vegetative & Generative Growth	Evaluation of the clones from Asian origin in a commercial substrate culture system & compared to “RZ Ritmo”

4.2 Plant material

The control of the experiments were the eggplant cultivars F_1 hybrids "RZ Adona" and "RZ Ritmo" from the Dutch seed company Rijk Zwaan, both with typical lilac colour and oblong shape for commercialization in the German market. As characteristic genotypes of Asian origin, were chosen eggplant genotypes from Vietnam. At the beginning of the experiments, breeds from different suppliers were used. From the governmental seed company in Hanoi the "1507" and "Cao San", as well as "Dok" from Saigon. Also, from the Vietnam Academy of Science (VAS) "VASI-5" was tested (see Table 4-2).

Table 4-2 Origin of the plant material

Solanum melongena genotypes	Breeding Company	Starting Plant Material for each experimental phase				
		I	II	III	IV	V
"RZ Adona" Control (2001-03)	Rijk Zwaan, Holland	Seeds [1]	Plantlets obtained *in vitro*[2]	1, 2	-	-
"RZ Ritmo" Control (2004-05)	Rijk Zwaan, Holland	-	-	-	1	1
"1507"	Seed Co. Hanoi, Vietnam	1	2	2	2	1, 2
"Cao San"	Seed Co. Hanoi, Vietnam	1	2	2	2	1, 2
"Dok"	Tropica Saigon, Vietnam	1	2	2	2	1, 2
"VASI-5"	VAS Hanoi, Vietnam	1	2	-	-	-

Commercial eggplant varieties have an upright growing habit and differ greatly in the form and colour of fruits. Traditionally, the lilac colour ("RZ Adona" and "RZ Ritmo") of the fruit has been accepted worldwide. Nevertheless, much more variability of this trait exists, especially, among the eggplant of Asian origin. Eggplant fruits of Asian genotypes are elongated, with a wide variation in colour and shapes (Fig. 4-1).

Fig. 4-1 Fruits of Asian eggplant genotypes

4.3 Propagation of the plant material *in vitro*

In this study tissue culture of the plant material (shoots) enabled the quick obtention of eggplant clones. Besides, experiments were designed to test the response of hypocotyl and cotyledon explants from five eggplant genotypes to different plant growth regulators at different concentrations.

4.3.1 Disinfection and explant selection

Surface-sterilization was performed by immersion of seeds into a solution of 3% (v/v) calcium hypochlorite with one drop of Tween 20 for 15 minutes in a shaker and then rinsed three times in the laminar air flow-cabinet with sterile distilled water, to remove any rests of the disinfectant solution in the plant material and to eliminate non-viable seeds from the trials; consequently those seeds that did not sink in the water were eliminated. During the manipulation in the laminar air flow-cabinet the seeds were kept in sterile distilled water to avoid desiccation.

4.3.2 Seed inoculation and culture medium

Following disinfection, each single seed was buried in the Germination and Multiplication Medium (GM&MM: Table 4-3) in a numbered test tube (25 x 150 mm) with 10 ml of half-strength MS medium (Murashige and Skoog , 1962; Table 4-3), supplemented with 2 % (w/v) sucrose and solidified with 0.7 % (w/v) agar-agar. The respective seed was manipulated in sterile conditions in the laminar air-flow cabinet. All the inoculations were made with sterile instruments. All reagents used for the tissue culture medium were obtained from SERVA with the exception of Tween 20 from FERAX. In all cases the products were of research grade purity. The culture media were adjusted to pH 5.8 prior to autoclaving at 120°C and at a pressure of 1.2 kg/cm^2 for 15 minutes. Cultures were maintained under photoperiod of 16h illuminated by lamps type OSRAM L 58W/25 and 8h darkness at 24°C $\pm$ 2. Subculture of the germinated seedlings took place every 5 weeks in the GM (Table 4-3) under sterile conditions. The multiplication was done by microcuttings of 1 cm each inoculating up to 6 microcuttings of the same clone in Erlenmeyer flasks (100 ml) covered with aluminium folios containing 20 ml of MM (Table 4-3). These clones were used for greenhouse evaluation.

Table 4-3 Reagents of the culture media for micropropagation of eggplant

Mineral composition	Concentration in mg/L	
	MS	GM&MM
Macroelements		
KNO3	1.900	Half strength of the MS chemical composition
NH4NO3	1.650	
MgSO4 * 7H2O	370	
CaCl2 * 2H2O	440	
KH2PO4	170	
Microelements		
H3BO3	6.2	Half strength of the MS chemical composition
MnSO4 * 4H2O	15.6	
ZnSO4 * 7 H2O	8.6	
Na2MoO4 * 2H2O	0.25	
CuSO4 * 5H2O	0.025	
CoCl2 * 6 H2O	0.025	
KI	0.83	
FeSO4 * 7H3O	27.8	
Na2 * EDTA	37.3	
Vitamines		
Biotin	-	0.2
myo-Inositol	-	100
Pyridoxine hydrochloride	-	0.2
Thiamine hydrochloride	-	2.5

4.3.3 Callogenesis and indirect shoot regeneration

The callus induction was started from cotyledon explants and 7 days old hypocotyl segments (3-5mm) of “RZ Adona”. The same tissue culture technique was tried on hypocotyl segments (3-5mm) of one week old seedlings of Asian eggplant genotypes “1507”, “Dok”, “Cao San” and “VASI-5”. The explants were laid in 100 X 10 mm Petri dishes with 10 ml of half-strength MS basal medium. The culture medium contained the salts (Table 4-3) of Murashige and Skoog (1962), 2 % (w/v) sucrose and solidified with 0.7 % (w/v) agar-agar. Cultures were incubated in the dark at 27°C. Callus induction on cotyledons explants of “RZ Adona” was induced with 2,4-D or NAA, at three different concentration (0,1 mg/L; 0,5 mg/L; 0,8mg/L). Callogenesis on hypocotyl segments of “RZ Adona” and Asian eggplant genotypes “1507”, “Dok”, “Cao San” and “VASI-5” was stimulated with NAA (0,5 mg/L) and TDZ (0,1 mg/L). Indirect organ regeneration was obtained in a hormone-free culture medium supplemented with activated charcoal (2,5 g/L). The manipulations of the explants and culture conditions as well as autoclaving of culture media were similar to those already described for micropropagation.

4.3.4 Rooting, hardening and physical factors

The eggplant shoots from micropropagation developed roots while being multiplied *in vitro* without need for further rooting enhancement treatment. The plantlets with *in vitro*-formed roots were removed from the culture media, the roots washed with tap water and transplanted in the acclimatising area to pots containing perlite-soil (1:1) mixture in a tray. The tray was covered with a plastic lid with holes every 20 cm to permit transpiration and hardening. The relative humidity was reduced slowly in the acclimatising area, where the temperature was 24± 2˚C and the 16-h photoperiod remained constant during acclimatisation period of two weeks.

4.3.5 Evaluated parameters and statistical analysis

Micropropagation

Records of the parameters seed germination frequency and multiplication factor per subculture were registered, aimed at selecting the clones to be planted in the greenhouse for further investigation. The seed germination percentage (SGP)[a] was evaluated, and the multiplication factor (MR)[b] of the genotypes was assessed after each subculture. Three subcultures, each considered a repetition, were included in the calculations. The experiment was performed using a completely randomized design and the data analysed with ANOVA, Tukey HSD and Kruskal-Wallis at 5 % significance level using statistical package SPSS version 11.

$$^{a}SGP = \frac{\text{Number of germinated seeds X 100}}{\text{Number of plated seeds}} \qquad ^{b}MR = \frac{\text{Number of multiplied seedlings X 100}}{\text{Number of established seedlings}}$$

Callogenesis and indirect shoot regeneration

It was registered the number of explants forming calli and subsequent regenerated shoots per genotype influenced by each culture medium. Twenty cotyledons of the high yield variety "RZ Adona" at a density of 5 explants per vessel and 2-5 hypocotyl segments per cultivar and medium were placed in *Petri* dishes. Responses to callogenesis were evaluated by recording the number and type of calli developed 5 and 10 weeks after the explants were plated. The results on shoot regeneration were obtained in the third evaluation after 3 months in culture. A visual analysis of the calli took place to define colour and texture of callus. The results about callogenesis and shoot regeneration are presented as percentage of the total number of calli and regenerated calli per genotype influenced by the concentration of the respective plant growth regulator in the callus induction medium, as well as the origin of the explant. The experiment was performed using a completely randomized design and the data analysed with

ANOVA, Tukey HSD and Kruskal-Wallis at 5 % significance level using statistical package SPSS version 11.

4.4 Molecular biology analysis (RAPD-PCR)

A preliminary study at DNA level of five eggplants genotypes was undertaken in order to assess the genetic diversity of the plant material. The experiments aimed at obtaining potential genetic markers for more accurate identification of the eggplant genotypes.

4.4.1 Plant material and DNA extraction

Total genomic DNA was extracted from 100 µg of fresh leaves of the seedlings cloned *in vitro* of the eggplant genotypes "RZ Adona", "1507", "Cao San", "Dok", "VASI-5" and an Albanian eggplant cultivar; and used as template for the PCR reaction. A DNeasy Plant Mini Kit (QIAGEN) was utilized for DNA isolation following the instructions of the producer (Att.VI).

4.4.2 DNA amplification

The DNA amplification was obtained by Polymerase Chain Reaction (Table 4-4) in a thermo cycler "Biometra" from BIOTRON (Japan), using 11 synthetic random 10 polymer primers (Table 4-4).

Table 4-4 DNA amplification reaction

DNA amplification				
Steps	Time	Temperature	Final step	
			Time	Temperature
initial denaturation	2 min	94°C	1 min	94°C
Denaturation	1 min	94°C	30 sec	35°C
Primer annealing	30 sec	35°C	7 min	72°C
Extension	1 min	72°C	-	
Number of cycles	45	-	-	

The reagents (Table 4-5) used for the mixture of the PCR reaction were manufactured by INVITEK (Germany).

Table 4-5 Sequence of Random Primers CARL-ROTH KIT-170

Random Primer number	Primer Sequence 5'→3' end	Random Primer number	Primer Sequence 5'→3' end
39	GTC CTA CTC G	50	ACG GTG CCT G
40	CTA CAC AGG C	53	GGA CTC CAC G
41	GTC CTT AGC G	55	CTG TAC CCC C
44	GAG TCA CTC G	56	TGC ACG ACC G
45	GTC CTGAGT G	57	CAG ACA CGG C
48	CAT CCC GAA C	-	-

4.4.3 DNA separation and RAPD-PCR analysis

The amplification products were separated in a 2 % (w/v) agarose gel in 1X TAE buffer visualized by staining with ethidium bromide and photographed under UV light. The polymorphic reproducible bands in the agarose-gels were scored as present (1) or absent (0) and identified with reference to the 200bp Standard Ladder DNA Marker (GenSura, USA). PCR reactions were performed three times to establish reproducibility of results.

Table 4-6 Reagents used in the PCR mixture for RAPD analysis on eggplant

Reagent	25 µl total volumen *	[1]10X PCR buffer without $MgCl_2$ [2] *S. melongena* genotype [3]dNTPs: Mix solution in mM of: 3'-deoxyadenosine 5'-triphosphate sodium salt (dATP) 3'-deoxycytidine 5'-triphosphate sodium salt (dCTP) 3'-deoxyguanosine 5'-triphosphate sodium salt (dGTP) 3'-deoxythymidine 5'-triphosphate sodium salt (dTTP) [4]*Thermus aquaticus* DNA polymerase (recombinant): is purified from an *E. coli* strain carrying a *T. aquaticus* DNA polymerase overproducing plasmid, catalyses 5'→3' synthesis of DNA, possesses low 5'→3' exonuclease activity, and is reported to have reverse transcriptase activity *total volumen of the reaction mixture
DNA template[2]	2.0 µl	
PCR-Buffer [1]	2.5 µl	
DNTP-Mix [3]	0.5 µl	
50 mM MgCl2	3.5 µl	
Taq DNA polymerase [4]	0.1 µl	
Primer-[5'→ 3'] 10 nucleotides	1.25 µl	
Sterile deionized H_2O	15.15 µl	

4.5 Experimental design, materials and equipment in the greenhouse

4.5.1 Type of greenhouse and irrigation system

The eggplant genotypes were grown in separated containers in a glass greenhouse and irrigated during 5 minutes with the nutrient solution (Table 4-7). A trickle irrigation system with 'Netafim' drippers was used with a capacity of 2 L h^{-1}. The irrigation frequency varied from 3 times in winter until 30 times per day in summer with 5 minutes irrigation for each cycle.

Table 4-7 Chemical composition of the nutrient solution for eggplant

Nutrient Solution (Voogts, 1998 modified)			
Element	Target (ppm)	Target (mmol)	Content in warter (ppm)
N	210	15.0	0
P	75	1.5	0
K	248	7.75	4
Ca	180	3.75	96
Mg	50	5	8
Fe	3	0.015	0
HCO3	80	80	199
S04	80	1.5	88
EC: 2-2.5		pH: 5.8-6.5	

4.5.2 Environmental conditions in the greenhouse

The climatic conditions were carefully controlled in the greenhouse during the whole experimental period. The mean temperatures (˚C) and relative humidity (%) as depicted in Fig. 4-2, as well as the global radiation (J/cm^2) as shown in Fig. 4-3, were monitored daily for each experimental phase. Eggplant is a crop with intense light consumption, for that reason lamps type PL-90 E (radiation power of 160 W/m^2) were used between 7:00 and 19:00 hours during the winter experiment, programmed to turn off when there were more than 10 Klux inside the greenhouse.

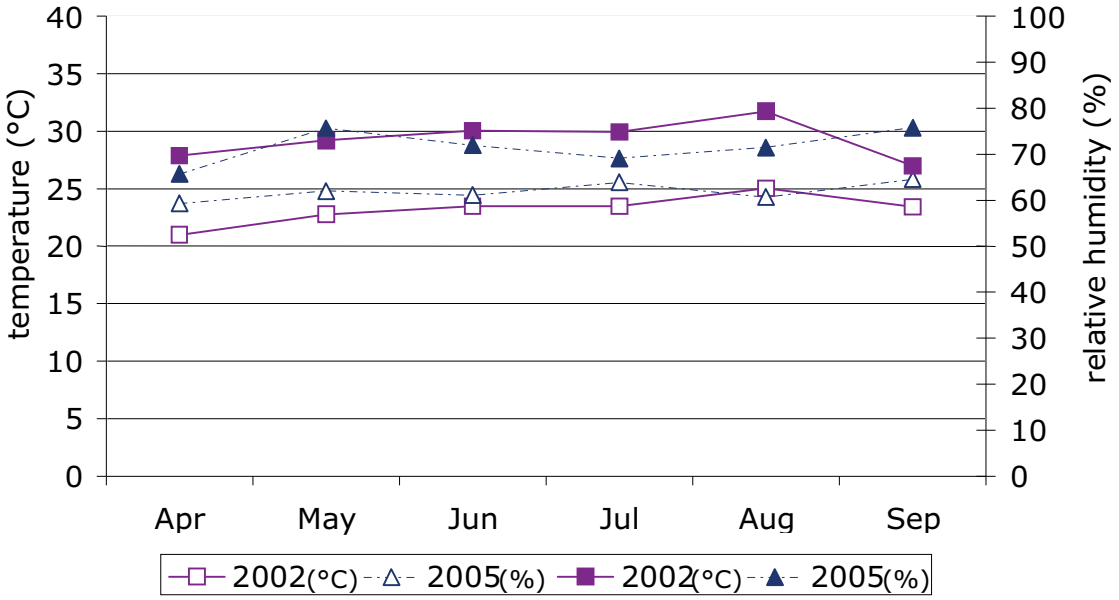


Fig. 4-2 Climatic conditions in the experimental area

In Fig. 4-2 are presented the mean air temperature (°C) and relative humidity (%) in the growing area in the summer cultivations of 2002 and 2005. The temperature represented by cubes ranged between the mean minimum of 21°C in 2002 and the mean maximum of 33°C in 2005. The mean relative air humidity simbolized by triangles fluctuated between 60% in 2002 and 80% in 2005.

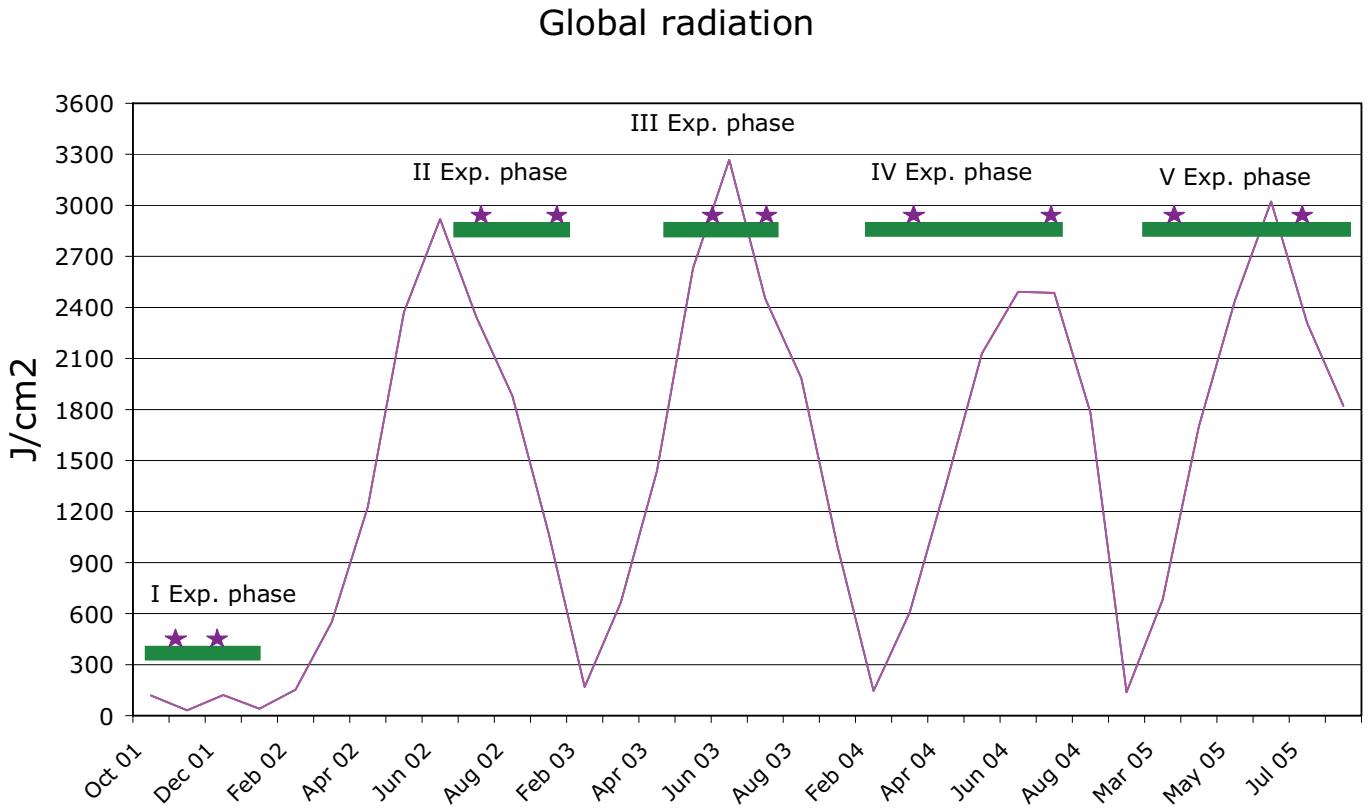


Fig. 4-3 Global radiation during the experimental years

In Fig. 4-3 are shown the irradiation (J/cm^2) during the vegetative growth represented by green rectangles, as well as for the flowering period indicated by the stars for each of the experimental phases. The light intensity (J/cm^2) fluctuated according to the season; lower values (<300 J/cm^2) were registered during the winter period (Oct.-Feb.) than in spring and summer (Apr.-Aug.), with values of light intensity that ranged between 1200 J/cm^2 and 2800 J/cm^2 to 2500 J/cm^2 in 2002 and 2004, respectively. The radiation reached values above 3000 J/cm^2 in the summers of 2003 and 2005.

4.5.3 Methodology and statistical methods

The eggplant seedlings were planted in a randomised block and the data analysed with ANOVA, Tukey HSD and Kruskal-Wallis at 5 % significance level using statistical package SPSS version 11. The sample number (n) varied for the different experiments, and different substrates were used (Table 4-8). The following parameters were registered weekly: leaf number, plant height (cm), flowering beginning, fruit set (%), fruit number and fruit fresh weight (g) per genotype, in winter and summer cultivations.

Table 4-8 Eggplant sample number for the greenhouse experiments

Evaluated plants (n)	Substrate	Evaluation period
30	org: "B_{700}" (Att. I)	10.2001-1.02
26	inorg: Perlite	
74	org: "B_{700}"	6-9.2002
31	org: "B_{700}"	5-8.2003
40	org: Peat slabs	2-8.2004
60	org: Peat slabs	2-8.2005

4.5.4 Collection of the experimental data

In the greenhouse, a week after transplantation started the evaluation of both, the vegetative and generative development of all genotypes. For the plantlets growing in perlite the evaluation period lasted 4 weeks, for that experiment was then stopped. The plant height was measured in cm from the soil surface to the highest bud. The length of leaves was measured from the leaf lobule to the tip, and the width between the two broadest horizontal extremes. The aim of these measurements was to find out a coefficient to calculate the leaf area. The different shape of the leaves did not make it easy to find a formula for the Asian genotypes. In most cases, the coefficient 0.6 was acceptable in order to have high correlation with direct measuring of the leaf area using an area scanner. The number of physiologically active leaves, the date of flowering beginning and the fruit number and fresh fruit weight in grams, were registered weekly for each plant of all genotypes. In the results, the fresh weight of the fruits is given in kg for practical reasons when drawing the respective figures.

5 Results and Discussion

In this study, the tissue culture of the plant material enabled the quick obtention of eggplant clones, which was the starting plant material for the experiments carried out. The treatments on **callogenesis** induction and subsequent **organogenesis** or indirect shoot regeneration gave insight in the response of **hypocotyl** and **cotyledon** explants from five eggplant genotypes to different plant growth regulators at different concentrations.

5.1 Cultivation of eggplant *in vitro*

The micropropagation of the plant material was carried out in three phases: first germination of the seeds (Table 5-1); followed by the establishment and cloning of the seedlings as part of the multiplication phase (Table 5-1), represented in the results of the 1st, 2nd and 3rd subculture (Fig 5-1).

5.1.1 *In vitro* germination

The seeds of the eggplant genotypes "RZ Adona" and those of Asian origin germinated with distinct results in the MS culture medium without PGR. However, no statistical differences were found in this parameter. All the seeds of "RZ Adona" (100%) germinated and the seedlings were successfully transferred to the multiplication phase. The germination frequency varied among the Asian group of plants from 95% for "VASI-5" and 96% for "1507" to 100% for both "Cao San" and "Dok" (Table 5-1).

Table 5-1 Germinated seeds and established seedling of eggplant *in vitro*

Eggplant Genotype	Germinated Seed Number	Germinated Seed %	Transferred shoots segments to Multiplication phase
"RZ Adona"	12 out of 12	100	12
"1507"	25 out of 26	96	27
"Cao San"	37 out of 37	100	61
"Dok"	37 out of 37	100	40
"VASI-5"	37 out of 39	95	46

Each seed of "RZ Adona" produced one seedling that was transferred to the multiplication phase, in turn the shoots of the Asian group of plants were often large enough and yielded up to 2 shoot segments in the first subculture (Table 5-1) that were used as explants in subsequent cultures.

Discussion

The results of this study showed that eggplant seeds germinate as well as eggplant nodal segments form shoots *in vitro* without the use of plant growth regulators (PGR) in the culture medium. The germination of the seeds of the investigated eggplant genotypes was achieved in the half-strength MS culture medium supplemented with 20 mg L^{-1} sucrose without PGR. Similar results were obtained by Taha and Tijan (2002) in the MS basal medium supplemented with 30 mg L^{-1} sucrose. The five eggplant genotypes cultured *in vitro* on the half-strength MS basal medium developed well and continued a normal growth after transplantation in the greenhouse. Studies carried out by other authors reported similar results, with the difference that the MS culture medium was supplemented with PGR (Kashyap *et al*., 2003).

5.1.2 *In vitro* multiplication

The multiplication factor, which indicates the efficiency of the micropropagation process and refers to the number of explants obtained per subculture from a single shoot, was monitored and is shown in the statistically different results of subcultures 1 to 3 (Fig. 5-1).

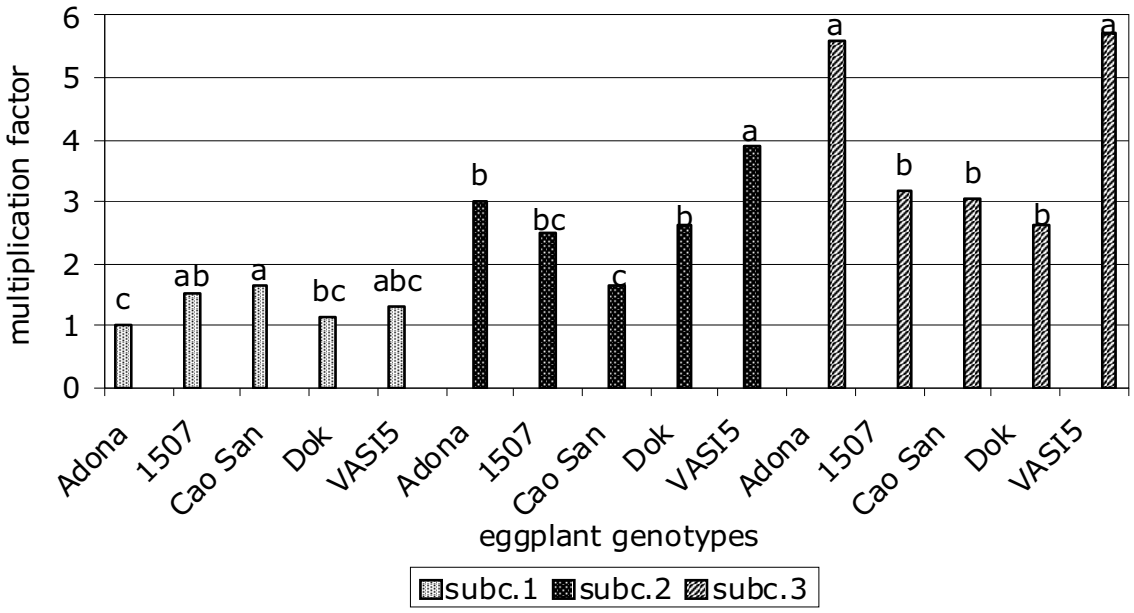


Fig. 5-1 Multiplication factor of eggplant genotypes in 3 subcultures every 6 weeks each in MS culture media (ANOVA, Tukey HSD & Kruskal-Wallis $P< 0.05$, different letters stand for statistical differences)

The multiplication factor, generally, increased with the number of subcultures, which took place every 6 weeks (Fig. 5-1). In subculture I "RZ Adona" (1) and "Dok" (1.13) had the lowest multiplication factor, even thought statistically comparable to that of "VASI-5" (1.3),

which in turn had similar result to "1507" (1.5) and lower than "Cao San" (1.64). In the 2nd subculture "VASI-5" (3.9) had the highest multiplication factor. The other genotypes had values that ranged between 1.6 and 3 that were statistically similar. In the 3rd one, "RZ Adona" (5.58) and "VASI-5" (5.7), obtained the highest multiplication factor, followed by "1507" (3,17), "Cao San" (3.03) and "Dok" (2.63) (Fig. 5-1).

Clones of eggplant seedlings (genotypes) were selected according to their phenotypical characteristics after *in vitro* cloning, to study their multiplication factor. Fig. 5-2 represents the average results in three subcultures of the multiplication factor of 3 clones of "RZ Adona", 10 clones of both "1507" and "VASI-5" as well as 9 clones of both "Cao San" and "Dok". In spite of the variability in the multiplication factor among the five eggplant clones, and especially within the Asian group of eggplant, the results were not statistically different.

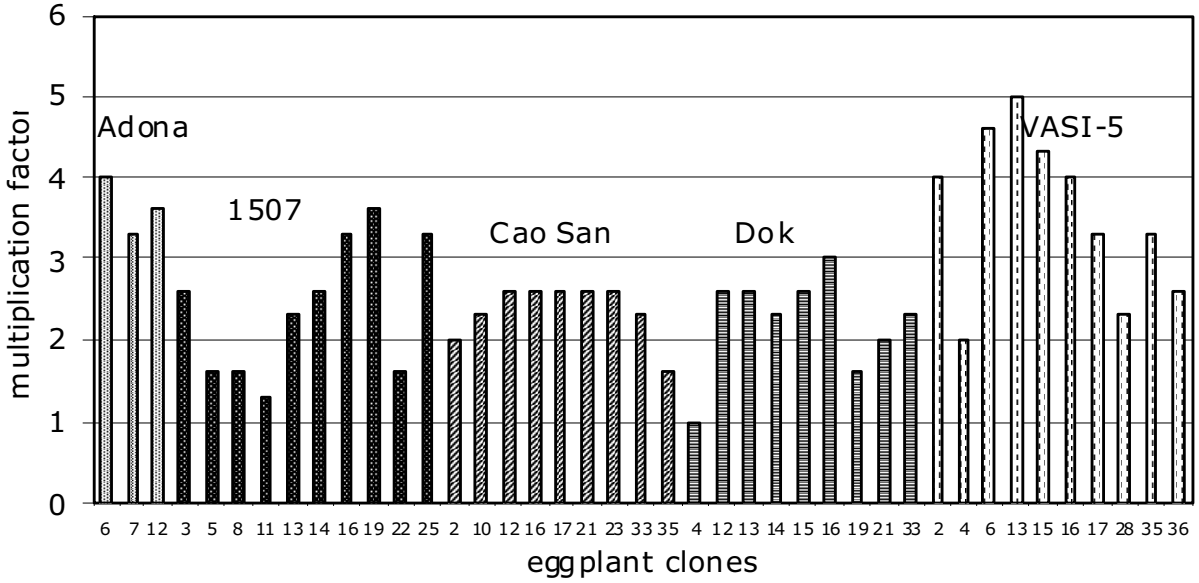


Fig. 5-2 Multiplication factor of eggplant clones after 6 weeks in MS culture media

The clones of "RZ Adona" (6, 7, and 12) had a multiplication factor that ranged between 3.3 and 4. The Asian group of plants showed variable results, with a multiplication factor that ranged between 1 and 5 in three subcultures, every 6 weeks each. There was variability on this parameter among the clones within the Asian genotypes as well: "1507" (1,3-3,6); "Cao San" (1,6-2,6); "Dok" (1-3); "VASI-5" (2-5) (Fig. 5-2).

Clones 6 (4,6), 13 (5) and 15 (4,3) of "VASI-5" had a higher multiplication factor than the rest of the clones of all genotypes. Another 7 clones of Asian origin, that is clones 16, 19 and 25 of "1507" as well as 2, 16, 17 and 35 of "VASI-5", had a multiplication factor in the same range (3,3-4) like "RZ Adona". A total of 28 clones of Asian origin had a micropropagation factor that ranged between 1 and 3 (Fig. 5-2).

Discussion

Micropropagation of plants is affected by many factors leading to differences in the multiplication factor. The genotype plays an important role on the multiplication factor between species as well as among varieties and clones within a single species (Orellana, 1998; Pinker, 2002). The number of subcultures may have either positive or negative effects (Orellana, 1998) on the multiplication factor. In this study, the micropropagation factor increased from subculture 1 to 3. Similarly, it has been reported that with the number of subcultures the number of shoots per explants tend to increase (Debergh and Maene, 1981; Rancillac *et al*., 1987). The multiplication factor *in vitro* can be improved by a correct management of the explant (Orellana, 1998) considering the time between subcultures and refreshing of the explants, the ability of the species to proliferate *in vitro*, the physical characteristics (volume, exchange capacity, transparency and size) of the culture vessel, type (liquid, solid or semisolid), composition and quantity of the culture medium, size and type of explant, growth habitat of the species *in vitro* and photoperiod (Pinker, 2002) as well as light intensity available in the growing areas.

5.1.3 Contamination of the eggplant material propagated *in vitro*

The *in vitro* multiplication of the established explants was successful, even though there were differences with regard to the susceptibility of the genotypes to contamination (Table 5-2).

Table 5-2 Contaminated explants after each phase of the micropropagation process

Genotype	Number of contaminated seed/seedling after			
	germination	Subc. I	Subc. II	Subc. III
"RZ Adona"	0	0	4	0
"1507"	1	4	0	0
"Cao San"	0	6	0	0
"Dok"	0	11	13	11
"VASI-5"	2	7	0	0

The number of contaminated explants during the micropropagation of the eggplant genotypes is shown in Table 5-2. In the germination phase one explant (seed) out of 26 from "1507" and 2 out of 39 from "VASI-5" became contaminated, which demonstrates that the external disinfection of the seeds was effective. In Subculture I, only seedlings of the Asian group of eggplants became contaminated: 4 seedlings out of 27 from "1507", 6 out of 61 from "Cao San", 11 out of 40 from "Dok" and 7 out of 46 from "VASI-5". In Subculture II, 4 seedlings out of 36 from "RZ Adona" became contaminated as well as 13 out of 66 from "Dok"; the genotype which had 11 out of 63 seedlings contaminated in Subculture III as well. In general, according to the results on Table 5-2, the Asian eggplant genotypes were more susceptible to

contamination than "RZ Adona". Contamination of the plant material occurred during the different steps of eggplant micropropagation. Interestingly, just few seeds became contaminated after they were buried in the germination medium, suggesting a successful external disinfection of the initial explants.

Nevertheless, both fungal and bacterial contamination was detected after the second and third subcultures of the plant material, especially among the Asian genotypes.

Discussion

In vitro contamination has been found to have many sources of origin (Alvarado, 1998); the initial explant being the most frequent (Cassells, 1991; Leifert *et al*., 1994; Stead *et al*., 2000), the environment in which the vitroplants are either manipulated or grown (Alvarado *et al*., 1993 and 1997; Stead *et al*., 2000), inefficient aseptic techniques (George, 1993; Weller, 1996; Alvarado *et al*., 1997), instruments and equipment in the laboratory (Boxus and Terzi, 1988; Leifert *et al*., 1994) and insects (Blake, 1988; George, 1993; Roca and Mogriski, 1993; Leifert *et al*., 1991 and 1994; Pype *et al*., 1996). Fungi, bacteria and yeast are among the most frequent contaminant micro-organisms found in the *in vitro* cultures (Alvarado, 1998). Contamination that occurs post-establishment may be caused either by bacteria concealed within the initial explant (endogenous) or by micro-organisms introduced when cultures are manipulated in the laboratory (Van den houwe and Swennen, 2000). Earlier reports have confirmed the possible survival of bacteria in covert or endophytic form in plant tissue cultures (Thomas, 2004; Alvarado, 1998). In the case of fungi, it is unlikely that they can be found latent in plant tissues (Alvarado, 1998; Leifert, 2000). A study conducted by Van den houwe and Swennen (2000) on 851 accessions of banana kept *in vitro* for extended periods of time that considered the bacterial species causing the cultures contamination, their typical features and habitat, and the fact that they were detected randomly in the plant material, suggested that this contamination was introduced during or after tissue culture establishment. Similar results were obtained by Leifert *et al*. (1989). Taking into account that the contamination of the eggplant genotypes, mainly those of Asian origin, was caused by either fungi or bacteria that appeared after the plant material was transferred to the multiplication phase, which occurred randomly in cultures maintained *in vitro* for months; and the fact that similar situations are described in the literature (Van den houwe and Swennen, 2000), it is most likely to be that the contamination encountered in the *in vitro* cultures of eggplant in this study was probably introduced during tissue culture manipulation. The possibility that the contamination was introduced *in vitro* with the initial explants cannot be excluded, but it may not necessarily mean that it is due to endogenic contamination, even though the latest cannot be ruled completely out.

5.1.4 Callogenesis and organogenesis of eggplant

In this study was compared in 2 experiments with distinct variants, the influence of 2,4-D, NAA and TDZ as Plant Growth Regulators (PGR) at different concentrations on the response to **callogenesis** and **organogenesis** of 5 eggplant cultivars ("RZ Adona", "1507", "Dok", "Cao San" and "VASI-5"). Besides, the origin of the explants, e.g. **cotyledon** and **hypocotyl**, was regarded as another parameter in this study.

Experiment I: **Callogenesis** and **organogenesis** of the eggplant cultivar "RZ Adona" were influenced by explant type as well as PGR and its concentration. The influence on calli formation of PGR 2,4-D and NAA at three concentrations (0,1; 0,5 and 0,8 mg/L) on **hypocotyl** and **cotyledon** explants of the eggplant cultivar "RZ Adona" was investigated. The combination of MS basal medium with 2,4-D and NAA (0,1; 0,5 and 0,8 mg/L), as suggested in previous investigations, resulted in the callus formation by the explants.

- Callogenesis of eggplant cultivar "RZ Adona"

"RZ Adona" had a positive response to callus induction, and differences were observed on the response to **callogenesis** according to origin of the explant as well as the concentration and type of the PGR. The statistical differences could not be proven, probably due to the high variability of the results (Table 5-3).

Table 5-3 Callogenesis (%) of "RZ Adona", influenced by explant type and PGR (n=20; $p<0.05$)

Explant	Explants with response after 5 weeks treatment	PGR					
		2,4 D (mg/L)			NAA (mg/L)		
		0,1	0,5	0,8	0,1	0,5	0,8
Cotyledon	callus	90	70	55	80	80	85
	spots of callus	10	30	45	20	20	15
	no callus	-	-	-	-	-	-
Hypocotyl	callus	80	90	100	100	100	100
	spots of callus	20	5	-	-	-	-
	no callus	-	5	-	-	-	-

Though, **cotyledons** formed calli under all teatments, and despite of the fact that 5 % of **hypocotyl** segments failed to form calli in one of the variants (0,5 mg/L 2,4-D), callus formation percentages were higher in **hypocotyl** segments than that in **cotyledon** explants. Different concentrations of NAA stimulated callus formation in 80 to 100 % of both explant types. While with 2,4-D in the culture induction medium the concentration effect was stronger and between 55 and 90 % of the explants could be stimulated to form callus. Only the variant with 0,8 mg/L 2,4-D induced callus in 100 % of the **hypocotyl** explants (Table 5-3).

- Organogenesis from callus of eggplant cultivar “RZ Adona”

The regeneration of plant organs (shoots) was achieved in the MS hormone-free culture medium supplemented with activated charcoal (2,5 mg/L), from callus of “RZ Adona”. The indirect regeneration of shoots is depicted in Fig. 5-3 from **hypocotyl** calli induced with 2,4-D or NAA.

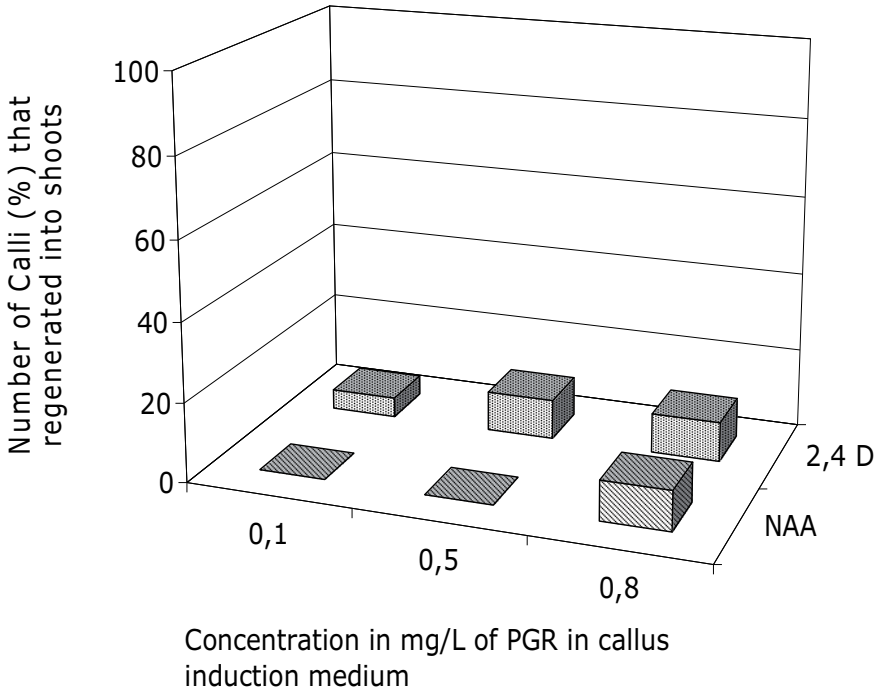


Fig. 5-3 Indirect shoot regeneration from hypocotyl callus of cultivar "RZ Adona"

The **hypocotyl** calli of “RZ Adona” had higher percent of shoot regeneration when the callus was induced with 2,4-D, than when the calli formed in the presence of NAA. 5, 10 and 10 % of calli coming from CIM I (0,1 mg/L 2,4-D), CIM II (0,5 mg/L 2,4-D) and CIM III (0,8 mg/L 2,4-D) respectively, regenerated plant organs. 10 % of the calli from CIM VI (0,8 mg/L NAA) produced shoots. Besides, the calli from CIM IV (0,1 mg/L NAA) and CIM V (0,5 mg/L NAA), did not regenerate plant organs at all (Fig. 5-3).

The results on indirect shoot regeneration from **cotyledon** calli are shown in Fig. 5-4.

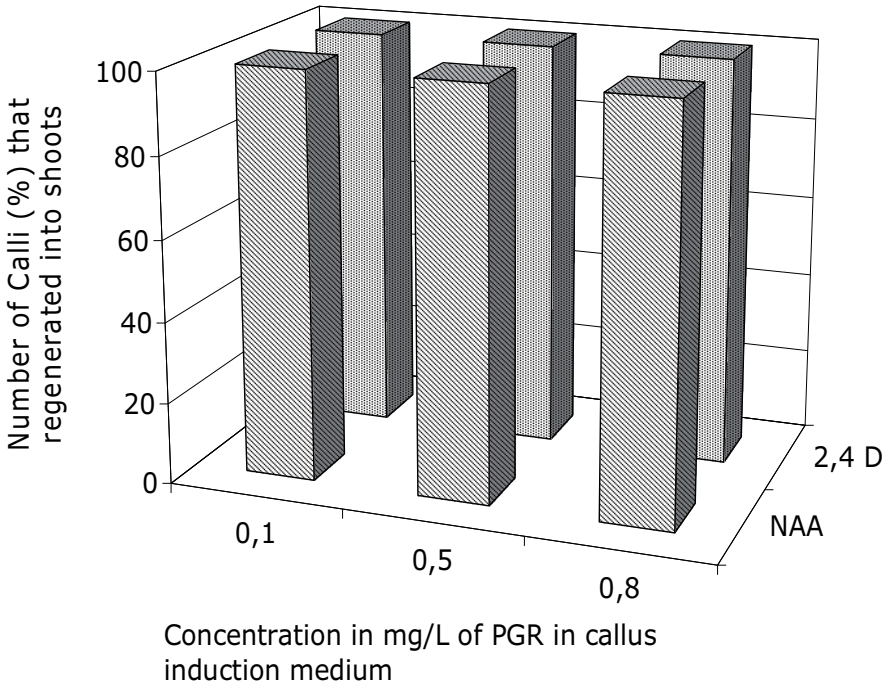


Fig. 5-4 Indirect shoot regeneration from cotyledon callus of cultivar "RZ Adona"

The regeneration of shoots of “RZ Adona” (Fig. 5-4) was achieved from **cotyledon** calli obtained with both, 2,4-D and NAA. Nevertheless, the phenotypical appearance of the regenerated shoots seems to have been influenced by the type and concentration of PGR. The shoots that were obtained from **cotyledon** calli of “RZ Adona” in the variants of CIM with 2,4-D developed into vitrificated plantlets, which often had curled leaves. While, those **cotyledon** calli that formed in the presence of NAA regenerated shoots that developed into plantlets with apparent normal phenotypes.

Experiment II: **Callogenesis** and **organogenesis** on **hypocotyl** explants from “RZ Adona” and four Asian eggplant genotypes, influenced by PGR and its concentration.

The eggplant genotypes “RZ Adona”, “1507”, “Dok”, “Cao San” and “VASI-5” formed calli (Fig. 5-5) under the influence of the plant growth regulators NAA (0,5 mg/L) and TDZ (0,1 mg/L).

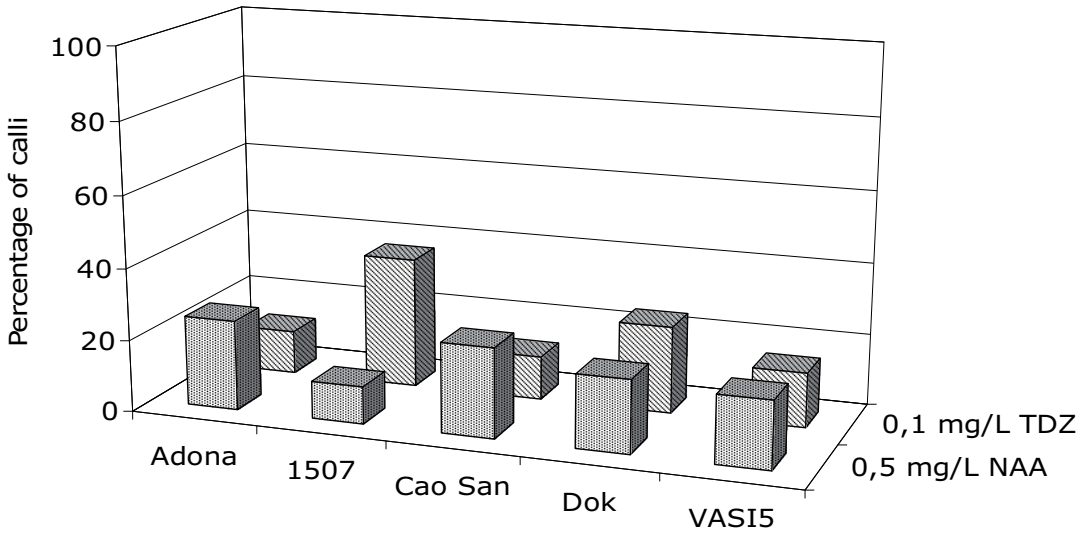


Fig. 5-5 Callogenesis on hypocotyl segments from different eggplant genotypes

The **hypocotyl** segments of the eggplant cultivar "RZ Adona" formed callus in the presence of 0,5 mg/L NAA (25 %) and 0,1 mg/L TDZ (12 %). Similar results were obtained for Asian genotype "Cao San". Eggplant genotypes "Dok" and "VASI-5" reacted similar to both callus induction media, with 20 and 24 % of calli induced by 0.5 mg/L NAA or 0,1 mg/L TDZ for the first genotype; as well as 19 and 15 % of calli formation influenced by either PGR for the latest, respectively. Asian eggplant genotype "1507", responded in a different manner than the other genotypes. It obtained higher percentage (36 %) of calli formation with 0,1 mg/L TDZ, and lower (10 %) with 0,5 mg/L NAA. In this experiment was observed that the reaction on **callogenesis** of the studied 5 eggplant genotypes differed, even though it could not be statistically proven (Fig. 5-5).

Also, the colour and consistency of the callus formed under the influence of the distinct PGR differed among eggplant genotypes (Fig. 5-6). In the presence of NAA, the **hypocotyl** explants from distinct eggplant types developed friable and creamy calli (Fig. 5-6 a, b). In the variant with TDZ the calli from **hypocotyl** explants were rather compact and often turning dark brown (Fig. 5-6 c).

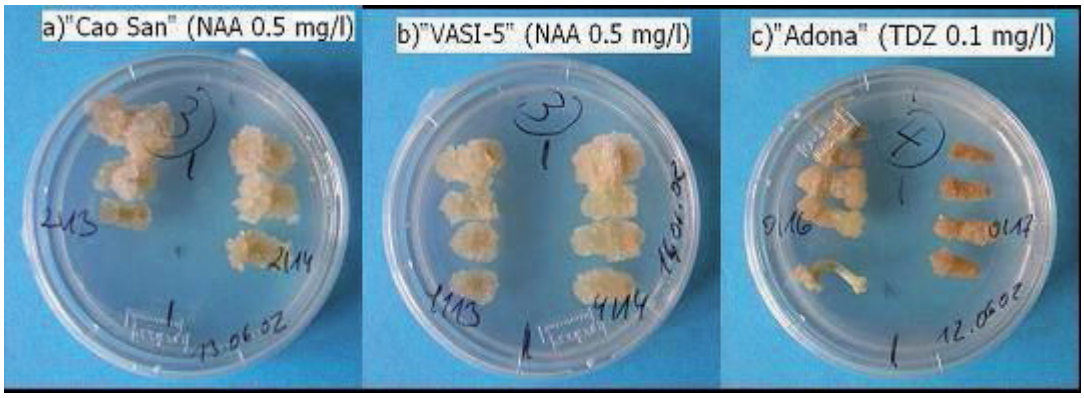


Fig. 5-6 Colour and consistency of calli from eggplant induced by NAA and TDZ

After callogenesis of the five eggplant genotypes was achieved, the **organogenesis** capacity of the calli was assessed (Fig. 5-7) considering the influence of each PGR present in the CIM. The evaluation took place after 20 days of growth with the calli exposed to light in a MS basal hormone-free medium supplemented with 2,5 mg/L of activated charcoal.

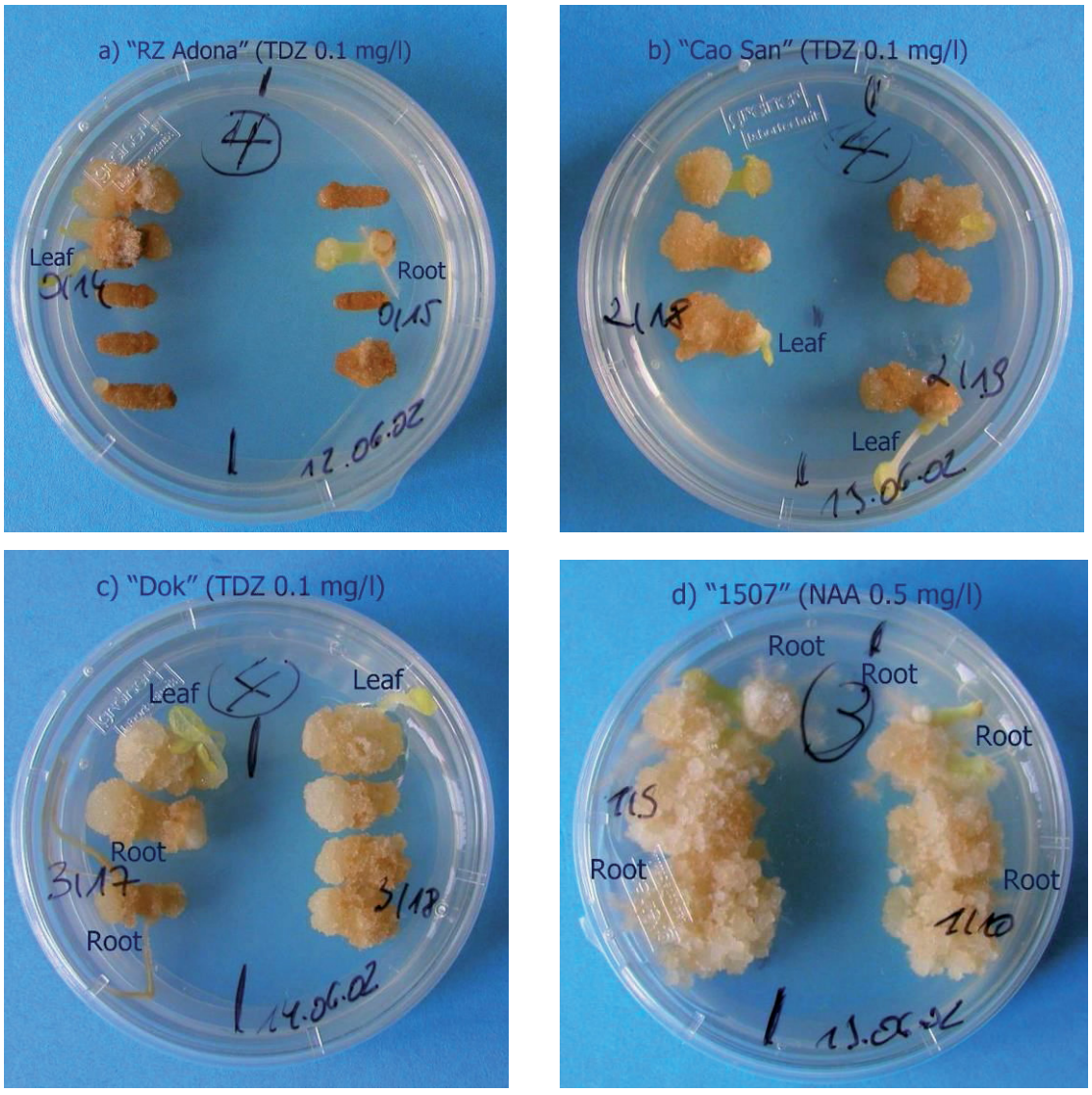


Fig. 5-7 Regeneration of root and leaf in eggplant from calli induced by TDZ and NAA

The regeneration of plant organs (shoots and roots) from **hypocotyl** calli of the 5 eggplant genotypes seemed to have been stimulated by the PGR used for calli formation. The calli from different eggplant genotypes induced in the presence of 0,1 mg/L TDZ regenerated into shoots in Fig. 5-7: a) "RZ Adona", b) "Cao San" and c) "Dok" as well as roots in a)"RZ Adona" and c)"Dok". NAA stimulated the regeneration of roots in "1507" (Fig. 5-7 d). However, indirect regeneration in a hormone free medium was hardly possible, from calli obtained with TDZ 0,1 mg/L. Calli of Asian eggplant genotype "Dok" (TDZ 0,1 mg/L) developed structures that seemed to have embryogenic characterists after inoculation to the hormone free regeneration medium.

Discussion

Callogenesis and organogenesis of eggplant have been thoroughly investigated (Allicho *et al*., 1982; Gleddie *et al*., 1983; Sharma and Rajam, 1995) using IAA, NAA and 2,4-D as plant growth regulators (PGR) at different concentrations (from 0,1 up to 10 mg/L) on explants of different origin (leaf, hypocotyls, cotyledons, etc). The results of these experiments are in agreement with those carried out by Magioli *et al*. (2000) and suggest a strong influence of explant type, genotype as well as type and concentration of PGR, on the explant response.

Callogenesis as well as organogenesis of eggplant and wild relatives has been obtained with the combination of different PGR and types of explants (Kashyap *et al*., 2003; Magioli and Mansur, 2005). The callus formation from hypocotyls of eggplant was reported with IAA, BA, Kn or Zn (Kamat and Rao, 1978); NAA or BA (Matsuoka and Hinata, 1979); 2,4-D (Allicho *et al*., 1982). Similar results were obtained from cotyledons and leaf explants of *Solanum melongena* (Allicho *et al*., 1982), *S. torvum*, *S. aculeatissimum*, *S. aviculare* and *S. gilo* (Gleddie *et al*., 1985) as well as both *S. torvum* and S. *Khasianum* (Kowalozyk *et al*., 1983) with 2,4-D, NAA, NOA, IAA, BA and Zn, as read in Magioli *et al*. (2000). Specifically, thidiazuron (TDZ) has proven to be very effective in inducing *in vitro* shoot regeneration of several species such as the kiwi fruit (Suezawa *et al*., 1988), apple (Fasolo *et al*., 1989), grape (Matsuta and Hirabayashi, 1989), pea (Bohmer *et al*, 1995), peanut (Kanyand *et al*., 1994), *Paulownia tomentosa* (Corredoira *et al*., 2008) and several woody species (Huetteman and Preece, 1993). TDZ produces high cytokinin-like activity in *in-vitro*-cultivated cells (Wang *et al*., 1986; Saxena *et al*., 1981) as read in Magioli *et al*. (2005). As read in Magioli *et al*. (2005) TDZ mechanism of action is partly related to the inhibition of cytokinin degradation by cytokinin oxidase, resulting in increased levels of endogenous cytokinin (Hare and Van Staden, 1994). In the study from Magioli *et al*., (2005) leaf and cotyledon were significantly more responsive than other explant types in the presence of TDZ. Besides, the highest rate of shoot development was observed on cotyledon-derived

calli maintained on MS medium supplemented with 0,2 μm TDZ, but they were short and failed to develop roots in response to several root inducing media. TDZ is reported to increase the levels of endogenous cytokinins (Hare and Van Staden, 1994) in Magioli *et al*. (2005). It is therefore possible as read in Magioli *et al*. (2000), that the inhability to form roots observed in these shoots was due to higher endogenous cytokinin levels. This idea is supported by the observation that shoots excised after subculture of calli on MS supplemented with 0,01 mm TDZ rooted at a frequency of 6% on half-strength MS supplemented with 0,6 mm IAA and 3% sucrose. Moreover, highly efficient root induction (70%) was achieved when calli were maintained on hormone-free medium before shoot excision for a period of 2 weeks after bud induction by TDZ. Plants which were produced in this manner were successfully transferred to soil and were phenotypically normal (Magioli *et al*., 2005).

5.2 RAPD-PCR analysis

In this study, RAPD-PCR analysis was expected to provide more insight on the genetic pattern of these plants (Karihaloo *et al*., 1995), and to detect minimal genetic differences (Prakash *et al*., 2002) among genotypes; information of great interest for the selection of parents for both hybridization and genetic breeding programmes of eggplant.

The amount of DNA gained from the different eggplant genotypes: I (Adona/$6_{a;b}$); II (1507/$5_{a;b}$) ; III (Cao San/$6_{a;b}$); IV (Dok/$15_{a;b}$) and V (VASI-$5_{a,b}$) was estimated to be enough for the PCR amplification reaction; based upon empirical observation of the result of the agarose gel electrophoresis (Fig. 5-8).

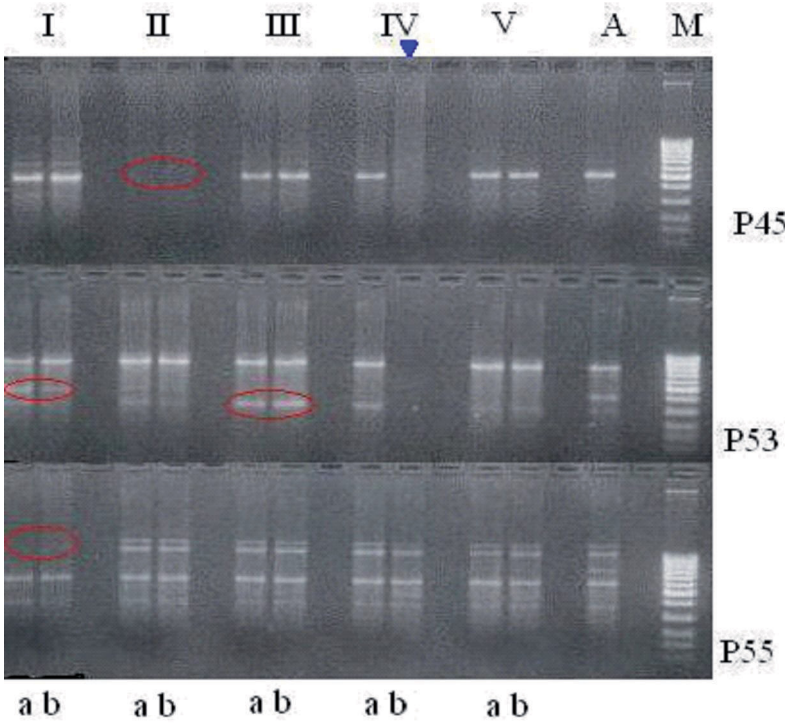
Fig. 5-8 Gel electrophoresis of DNA extracted from different eggplant clones (I (Adona/$6_{a;b}$); II (1507/$5_{a;b}$) ; III (Cao San/$6_{a;b}$); IV (Dok/$15_{a;b}$) and V (VASI-$5_{a,b}$), where a, b refer to DNA samples from distinct plants of the same clone)

The DNA extracted with the Kit Qiagen method was homogenous and not degraded (Fig. 5-8) and it was successfully amplified (Fig. 5-9) by the *Taq* polymerase as well, which indicated the consistency of the protocol and that no contaminants were present in the PCR reaction. The eggplant DNA samples were analysed with random primers 39, 40, 41, 44, 48, 50, 56 and 57. In all cases no differences were found in the RAPD-DNA profile of the clones. The PCR reaction was adjusted by changing the concentration of various components of the reaction mixture. Best results were achieved when the concentration of $MgCl_2$ was increased to 3,5 μl

combined with 2.5 µl 10X buffer without $MgCl_2$, plus 1.25 µl of the primer. Preliminary optimization experiments served to optimize the conditions for the PCR reaction. They included changes in the concentration of magnesium and the amount of DNA added per reaction aiming at the obtention of a reproducible pattern of the eggplant genotypes DNA, a similar approach was reported by Prakash *et al*. (2002) and by Pérez *et al*. (1998b). Also, the primers generating more reproducible fragment patterns and the higher differences at the DNA level were selected, which saved time, cost and helped to reject primers not informative for the analysis. Besides, once identified, the selected primers can also serve to evaluate other genotypes in future studies (Prakash *et al*., 2002).

Nevertheless, some irregularities were detected. Presumably, DNA quality problems (Wink, 1994) were present. DNA samples "a" and "b" of clone "Dok"/15 reacted differently from primers 45, 53 and 55 (blue dot on Fig. 5-9), although both DNA samples have a common origin, were extracted at the same time, under the same conditions, using the same procedure and were present in the electrophoresis that confirmed the DNA presence after its extraction (Fig. 5-8). Fig. 5-9 shows that in the reaction with P55 both DNA samples of clone "Dok"/15 can be clearly seen, while in the reaction with P45 and P53 DNA the sample is absent.

A preliminary study was carried out using the RAPD-PCR technique, to check the potential differences among the genotypes and pre-selected clones at the DNA level.



I (Adona/$6_{a,b}$) II (1507/$5_{a,b}$) III (Cao San/$6_{a,b}$) IV (Dok/$15_{a,b}$) V (VASI-$5_{a,b}$)
A (Albanian *S. Melongena* cv) M (DNA Ladder 200bp)
a,b refer to DNA samples from distinct plants of the same clone

Fig. 5-9 RAPD-PCR analysis of eggplant clones

DNA of "RZ Adona" and eggplant clones of Asian, as well as one clone of Albanian origin was used as template for the PCR reaction. Fig. 5-9 represents the eggplant clones RAPD-PCR pattern generated by random primers 45, 53 and 55.

Indication of genetic variability (Fig. 5-9), even though at low level, is that the genotypes reacted differently when exposed to the same primer. In the PCR reaction with Primer 45, Asian eggplant "1507" did not show any bands as the other genotypes did; "RZ Adona" and "Cao San" formed lower bands with Primer 53 that no other genotype had and "RZ Adona" did not show the upper bands in the reaction with Primer 55, as oppose to the rest of the genotypes.

A preliminary screening helped to select the primers generating more reproducible fragment patterns and the higher level of differences in the eggplant clone DNA profile (Table 5-4).

Table 5-4 RAPD amplified fragments of eggplant generated by 3 random primers

Primer	Sequence 5'→□3'	Total number of amplified fragments
45	GTC CTG AGT G	5
53	GGA CTC CAC G	10
55	CTG TAC CCC C	17

As indicated in Table 5-4 the number of bands generated by each primer differed, going from 5 for Primer 45, to 10 for Primer 53 and up to 17 for Primer 55.

The total number of amplified fragments per genotype differed as well, as shown in Table 5-5.

Table 5-5 Amplified fragments per genotype for primers 45, 53 and 55

Primer / Genotype	45	53	55	Number of amplified fragments per genotype
I: "RZ Adona" (6a/b)	1	2	1	4
II: "1507" (5a/b)	0	1	3	4
III: "Cao San" (6a/b)	1	2	3	6
IV: "Dok" (15a/b)	1	2	3	6
V: "VASI-5" (6a/b)	1	1	3	5
A: "Albanian" *S. melongena*	1	2	4	7

"RZ Adona" formed between 1 and 2 bands for a total of 4 countable bands in its reaction with the random primers 45, 53 and 55. The number of amplified fragments of the Asian clones ranged from 0 up to 3 countable bands. "1507" formed up to 3 bands per reaction for a total of 4 amplified fragments. A total of 6 fragments were present in both, "Cao San" and "Dok"; and 5 in "VASI-5". The "Albanian" eggplant cultivar showed most of the bands that were amplified in the Asian genotypes as well as different ones; and registered a total of 7 countable bands (Table 5-5).

Discussion

The results from the RAPD-PCR analysis of 5 clones of *Solanum melongena* showed small differences among the genotypes.That is in agreement with results obtained by Karihaloo and Gottlieb (1995) and Karihaloo *et al*. (1995), who concluded that *S. melongena* has very little polymorphism confined to a small number of accessions. Even though, those genotypes are highly similar from the genetic standpoint, they possess interesting agronomical features that are described in subchapter 5.3 "Cultivation of eggplant *in vivo*" of this work. This makes them attractive for commercialisation. It should be considered that the genetic pattern within the genus *Solanum* is quite similar (Collonnier *et al*., 2001; Kashyap *et al*., 2003; Sękara *et al*., 2007). Moreover, isozyme analysis showed that *S. melongena* has very little polymorphism (Lester and Hasan, 1991; Karihaloo *et al*., 1995), even for species that are thought to have originated in different places, and whose morphological features are distinct (Nunome *et al*., 2001). RAPD assays has been useful to identify duplicate accessions in wheat germplasm collections (Cao *et al*., 1998), to facilitate early detection and elimination of dwarfs from batches of micropropagated banana (Damasco *et al*., 1996), to check genetic variation of *Sericea lespedeza* (Sundberg *et al*., 2002) as well as to distinguish *H. rosa-sinensis* cv. 'Brilliant Red' from *H. syriacus* cv. 'Aphrodite' in the callus stage or juvenile plant (Jenderek *et al*., 1997).

5.3 Cultivation of eggplant *in vivo*

The investigation in the greenhouse focused on the influence of the substrate on the vegetative development, the effect of the cultivation period and the microclimate in the experimental area as well as the genotype, on the vegetative and generative growth of the eggplant genotypes. The investigated eggplant genotypes were characterized qualitatively and quantitatively concerning plant and morphology, as well as the yield per plant (fruit number and fruit load in kg) of the eggplant genotypes.

5.3.1 Influence of the substrate on the vegetative development of the genotypes

The vegetative growth of both "RZ Adona" and the Asian eggplant ("1507", "Cao San", "Dok", "VASI-5") genotypes was influenced by the type of substrate in the greenhouse. Fig. 5-10 shows the morphological differences among the eggplant genotypes in the first 4 weeks of the growing period in the winter experiment. The numbers show a clear difference of the stem height (Fig. 5-10) and the leaf number (Fig. 5-11) between the plants growing in the organic substrate and those in perlite. In both parameters the figures refer to the average value per plant.

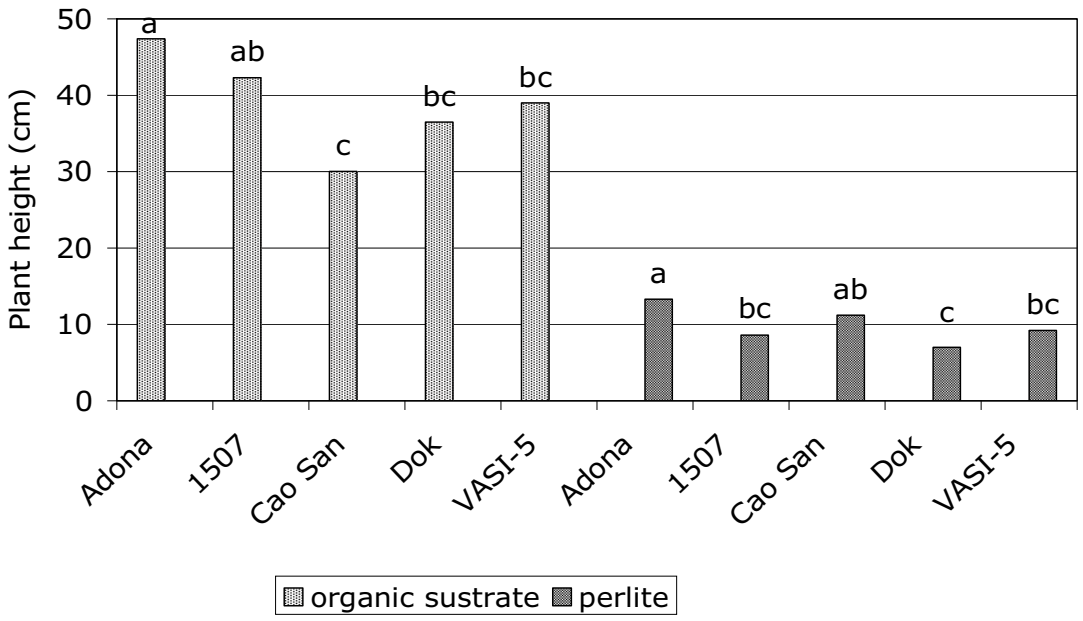


Fig. 5-10 Plant height (cm) of eggplant grown in winter influenced by organic substrate and perlite (Tukey HSD P< 0.05, different letters stand for statistical differences)

In both organic and inorganic substrate “RZ Adona” grew slightly faster than the Asian group of plants during the winter season of 2001-02. The height of the stem gave statistically different results among the eggplant genotypes, after 4 weeks of the growing period independently of the type of substrate. The eggplant seedlings reached from 30 to 47.4 cm of height within one month growing in the organic substrate whereas those in perlite grew from 7 to 13.3 cm toll . In the organic substrate Asian genotype “1507” (42.3 cm) had similar result like “RZ Adona” (47.4 cm), while “Dok” (36.5 cm) and “VASI-5” (39 cm) had intermediate values and “Cao San” with 30.02 cm had the shortest stem. In perlite “Cao San” (12.2 cm) grew almost as high as “RZ Adona” (13.3 cm), followed by “1507” (8.6 cm) and “VASI-5” (9.2 cm). “Dok” (7 cm) had the shortest stem (Fig. 5-10).

Also the number of leaves produced was affected by the type of substrate (Fig. 5-11).

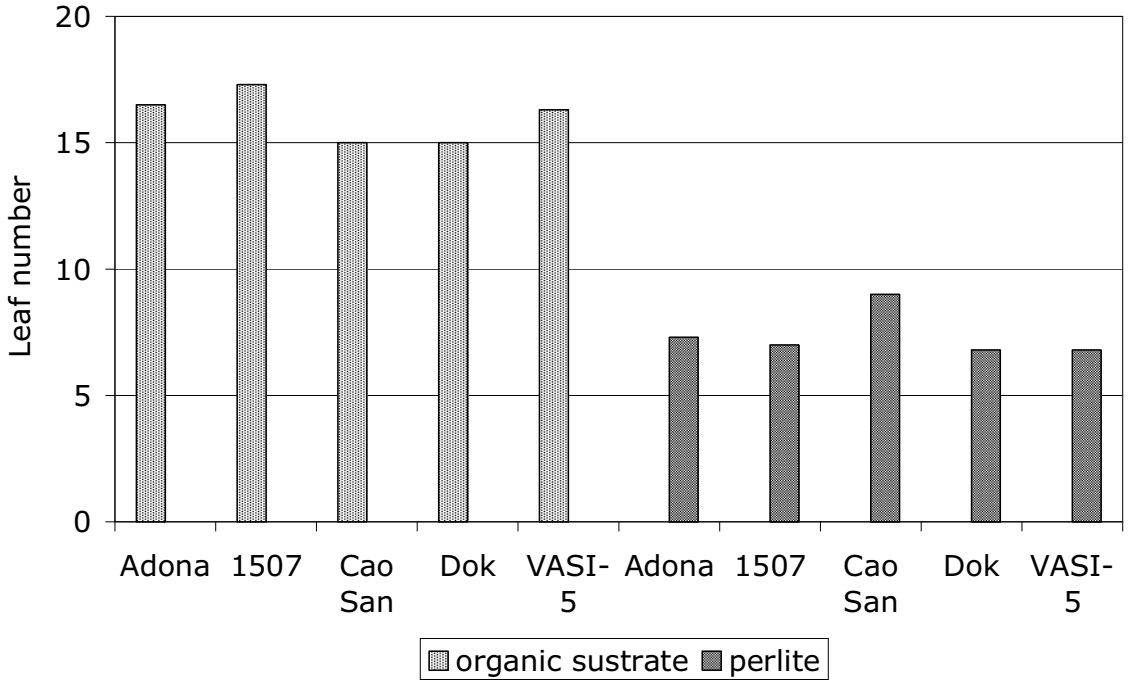


Fig. 5-11 Leaf number per plant cultivated on organic substrate and perlite during 4 weeks in the greenhouse in winter (ANOVA, $P< 0.05$)

The eggplants cultivated on organic substrate B_{700} (Att. I) developed double number of leaves (15 to 17) than those cultivated on perlite (7 to 9 leaves) after completing 4 weeks of vegetative growth in the greenhouse. Yet, the described results could not be proven statistically, suggesting in this case a much larger sample number.

Discussion

The cultivation of the eggplant genotypes "Adona", "1507", "Cao San", "Dok", "VASI-5" was successful under greenhouse conditions. The eggplants seedlings in this study developed better in the organic substrate B_{700} with than in the inorganic substrate perlite (Fig. 5-10, Fig. 5-11). The opposite was reported by Hahn *et al*. (2001), who stated that better results on vegetative and generative development of horticultural species were obtained in rockwool and perlite than in organic substrate. Soilless culture contributes to the improvement of plant growth, yield and earliness (van Winden, 1988) and provides an important alternative to soil cultivation system in greenhouse crop production; from both the phytosanitary and the economical viewpoints (Böhme, 1995; Hahn *et al*., 2001; Tüzel *et al*., 2001; Bougoul *et al*., 2005). Hahn *et al*. (2001) first micropropagated and then transplanted under greenhouse conditions two gerbera cultivars to soil and both, organic and inorganic substrates. The plants in soilless culture gave better results on morphological and generative traits, including earliness or first flower stalk formation than those in soil cultivation system. Generally, inert substrates have strong capillary action; specifically perlite is very porous as well, holds 3-4

times its weight of water (Tüzel *et al*., 2001) and keeps roots well aerated and watered (Olympios, 1992). Organic substrates that include peat in their composition have high cation exchange capacity (Verdonck, 1991) as well as high water holding capacity (Resh, 1991; Tüzel *et al*., 2001). Besides, organic substrates offer advantages in terms of ecology, crop yields being as good as in the conventional inert substrates (Böhme, 1994). Many aspects are contemplated when choosing substrates for hydroponic culture, being ecological and economical issues as well the major priorities. In the case of perlite can be used after disinfection for several years, has good structural stability and is available at low cost. Although the supply and disposal (compost) of the materials is not problematic, there are reservations about the use of compound organic substrate in terms of phytosanitary effects and variable composition (Böhme, 1994).

5.3.2 Morphological characteristics of the eggplant genotypes

In the first two experiments under greenhouse conditions, the eggplant genotypes of Asian origin were observed and compared among them, and with the high yield variety "RZ Adona", in order to select new eggplant clones suitable for greenhouse cultivation conditions. The following results show the characteristics of the vegetative growth of these plants in the greenhouse, both in a winter and a summer season. During the winter 2001-02 (Fig. 5-12) and the summer 2002 (Fig. 5-13) experiments the total number of leaves and the final stem height in cm were computed per plant to obtain the average value of these two parameters per genotype. The height of the stem was measured in cm from the surface of the substrate to the highest shoot.

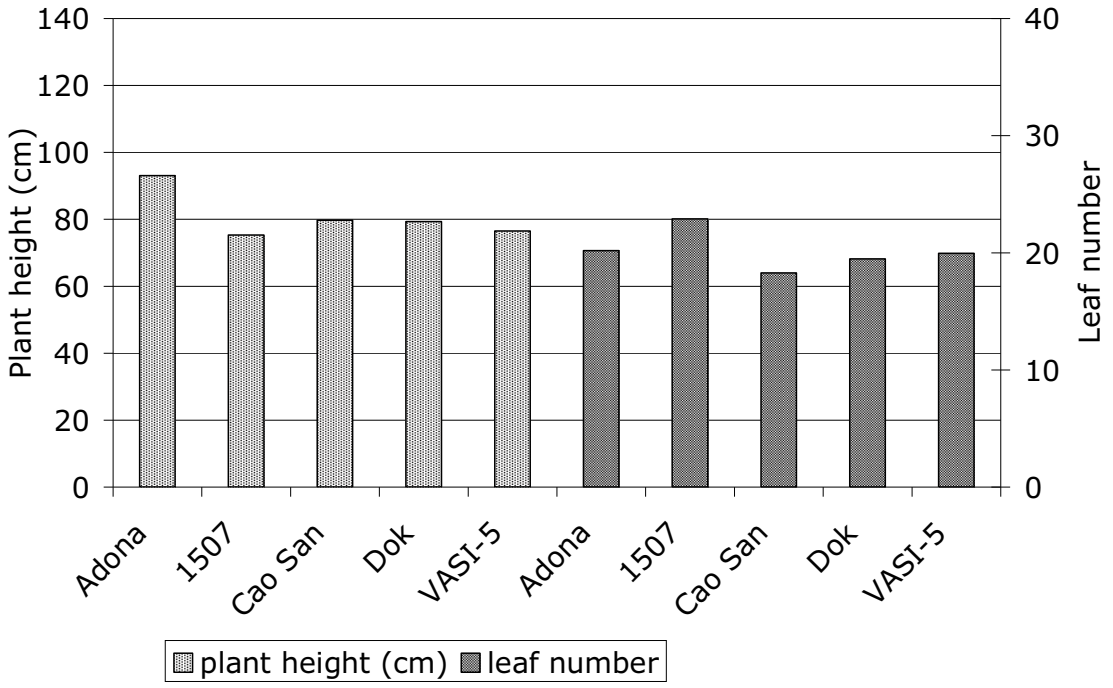


Fig. 5-12 Vegetative growth of eggplant on organic substrate in the greenhouse in winter cultivation (ANOVA, Tukey HSD& Kruskal-Wallis, P< 0.05, no significant differences)

In winter (Fig. 5-12) “RZ Adona” (93 cm) developed slightly faster and a higher stem than the Asian group of plants that grew from 75 to 80 cm. Interestingly, the Asian genotype “1507” (23) produced more leaves than “RZ Adona” and the other eggplants of Asian origin that averaged between 18 to 20 leaves per plant. The final stem height of “1507”, “Cao San”, “Dok” and “VASI-5” averaged from 75 to 80 cm; and “RZ Adona”, “Cao San”, “Dok” and “VASI-5” produced between 18 and 20 leaves on average during the winter period. But the observed morphological differences in the winter cultivation were not statistically significant, probably, because the sample number was not large enough.

The phenotypic evaluation of the genotypes in the summer experiment of 2002 (Fig. 5-13) resulted on statistically different values for both the plant height and the leaf number.

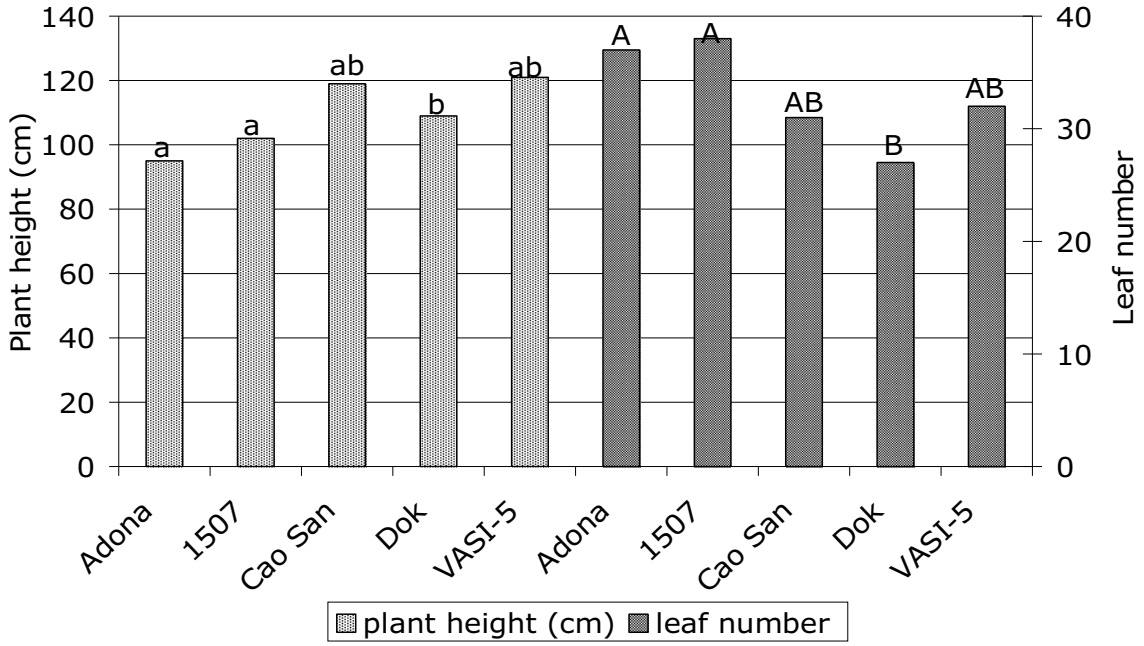


Fig. 5-13 Vegetative development of eggplant in summer cultivation under greenhouse conditions (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

In the summer cultivation of 2002 (Fig. 5-13) “RZ Adona” (95 cm) grew just 2 more cm than in the winter experiment (Fig. 5-12); it should be emphasized that the Asian genotypes had a better vegetative development in summer than in winter; as a consequence, the largest stems were developed by “VASI-5” (121 cm), and “Cao San” (119 cm). “RZ Adona” (37) and “1507” (38) developed a greater number of leaves than “Dok” (27), whereas “VASI-5” (32) and “Cao San” (31) obtained intermediate results of this parameter.

Additionally, the length and width in cm of each leaf were measured per plant to establish the mean value of these morphological traits per genotype (Fig. 5-14).

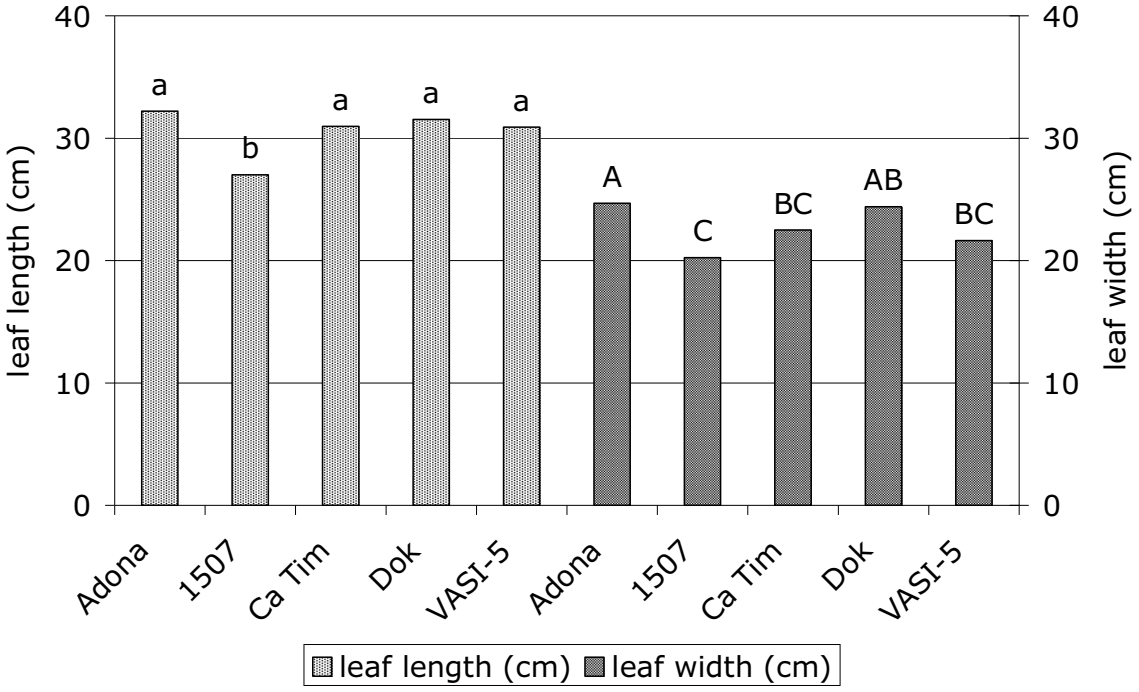


Fig. 5-14 Leaf dimension of eggplant grown in the greenhouse in summer (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

The length and width of leaves (Fig. 5-14), in general, brought significantly different results between "RZ Adona" and the Asian eggplant genotypes. Specifically, Asian genotype "1507", had the shortest (26 cm) and the narrowest (20.2 cm) leaves. The leaf length of "RZ Adona" and Asian genotypes "Cao San", "Dok" and "VASI-5" was similar and averaged 32.2, 31, 31.5 and 31 cm, respectively. With 25 cm "RZ Adona" produced the widest leaves followed by Asian eggplant "Dok" (24.4 cm), "Cao San" (22.5 cm) and "VASI-5" (21.6 cm).

Discussion

Light, temperature, humidity and CO_2 concentration are the major factors affecting plant growth. All-year-round crop production is possible in a controlled environment where the factors influencing growth can be monitored systematically (Park and Lee, 2001), and natural plant habitat simulated. Therefore, production in greenhouses becomes more popular than in the past, yet the key concept is to accelerate plant growth and the main objective is to achieve maximum yield per unit area. However, the relationship between climate conditions in greenhouses and the growth, development and productivity of crops requires special attention (Cemek *et al*., 2005). In this study "RZ Adona" had a similar vegetative development in winter and summer, while the Asian eggplants had a more vigorous growth in summer. This result suggests a stronger dependency on environmental factors, e.g. light intensity, for better vegetative growth of the Asian eggplant genotypes. Many literature reports are in agreement with the results of this investigation and focus on the influence of light on plant morphology

and development. Cemek *et al*. (2005) and Liu and Heins (1997), report that light determines crop growth. Challa and Schapendonk (1984), generally assume that the loss of light will lead to a proportional loss in yield. Also, the relative growth rate increase with higher light integrals due to an increase in whole plant photosynthesis and net assimilation rate, although increasing light integral affects differently the morphology of tomato and carnation (Bruggink, 1992). It should be noticed, however, that the photosynthetic response to light reaches a saturation point which varies between species (Moe, 1992). The effect of environmental factors, e.g. light (radiant energy), on plant growth and development is known. Radiant energy influences photosynthesis and therefore, plant biomass production (Liu and Heins, 1997). Oyaert *et al*. (1997) studied the influence of light quality and quantity on the growth of *Coleus*. They demonstrated the influence of the spectrum on plant growth. In agreement, Decoteu *et al*. (1997) postulated the influence of light on plant morphology and like Oyaert *et al*. (1997), reported the advantage of using light quality to develop an environmentally safe alternative to many conventional chemical growth regulator applications.

Qualitatively (Table 5-6), the characterization of the plant material took place in the greenhouse during the cultivation of eggplant from year 2001 to 2002 considering the guidelines in "DESCRIPTORS FOR EGGPLANT" (IBPGR, 1988). According to the original description by the producers of the investigated eggplant genotypes, these are F_1 *Solanum melongena* plants. In this study we found that the cultivars "RZ Adona" and "RZ Ritmo" (Fig. 5-15) were indeed very stable with no variation in fruit shape and colour. In experimental phases I, II and III from 2001 to 2003 "RZ Adona" served as control, and for the experimental phases IV and V "RZ Ritmo" did. There was variation among the Asian plants regarding fruit colour and shape (Fig. 5-16).

Fig. 5-15 Fruits of eggplant cultivar "RZ Ritmo"

Fig. 5-16 Fruit shape and colour of Asian eggplants: “Dok”; “Cao San” and “VASI-5”

Table 5-6 Qualitative morphological characterization of 5 eggplant genotypes (after guidelines of IBPGR, 1988)

Morphology of Eggplant Genotypes					
Characteristics	Control	Asian Genotypes			
	“RZ Adona” F_1	“1507” F_1	“Cao San” F_1	“Dok” F_1	“VASI-5” F_1
Plant					
Growth habit	upright	upright	upright	upright	upright
Stem colour	green	greenish-violet	greenish-violet	greenish-violet	green
Stem-prickles	yes	seldom	seldom	seldom	seldom
Leaf					
Blade lobbing	strong	weak	intermediate	strong	strong
Leaf-prickles	few	Intermediate	Intermediate	intermediate	intermediate
Flower					
Inflorescence	simple	cyme	cyme	cyme	cyme
Colour	light violet	light violet	light violet	light violet	light violet
Calyx-prickles	few	intermediate	intermediate	intermediate	intermediate
Fruit					
Shape	twice as long as broad	several times as long as broad	three times as long as broad	slightly longer than broad	three times as long as broad
Colour at com. harvest	purple black	striped lilac	striped lilac	striped lilac	striped white-lilac
Fr-calyx-prickles	very few	few	few	few	few

5.3.3 Generative development of the eggplant genotypes

The main advantage of greenhouse production systems is that it allows all-year-around production. In temperate regions, nevertheless, light becomes a constraint for cultures like eggplant that have a high demand for solar radiation. Therefore, beginning of the flowering period, the numbers of flowers as well as percentages of fruit set were among the decisive parameters for selection of suitable eggplant genotypes to be tried out in a greenhouse production system.

The parameters mentioned above for assessing the generative development were recorded for each genotype and clone in three experimental years (Winter 2001-02, Summer 2002 and Summer 2003). Those physiological and economically relevant parameters were the most important ones for selection of the eggplant clones that reached the last two experimental phases (Summer 2004 and Summer 2005).

- Beginning of the flowering period

The character of beginning of the flowering period is thought to be quite relevant for the selection purposes of new aubergine genotypes to be grown in temperate regions, where light is a constraint from the physiological and economical viewpoints for the production of this vegetable in the greenhouse.

In this work, the percentage of flowered plants per genotype (Fig. 5-17, Fig. 5-18) and clone (Fig. 5-19) was calculated considering the date of opening of the first flower for every plant. Thus, those genotypes and clones with a high percentage of plants with the first mature flower like the control “RZ Adona” that marked the beginning of the flowering period were regarded as early blooming genotypes. Consequently, those with a considerable percentage of plants that produced the first flower one or even two or more weeks later than the control were considered as late blooming genotypes/clones.

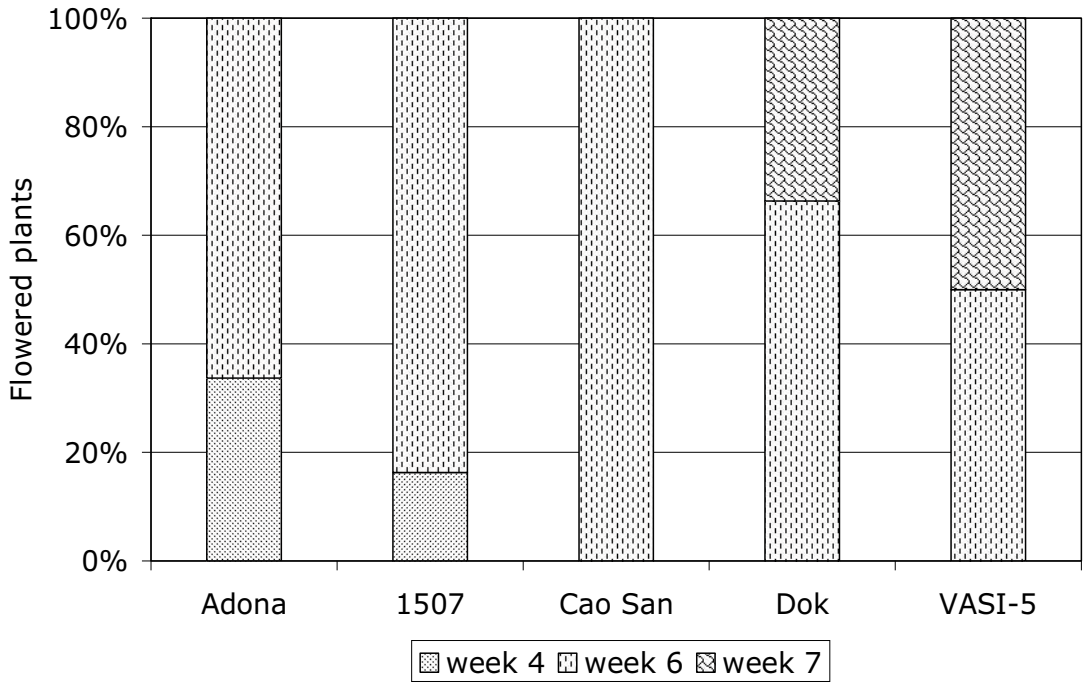


Fig. 5-17 Beginning of the flowering period of eggplant in winter in the greenhouse

In the winter experiment (Fig. 5-17) “RZ Adona” and “1507”, started flowering one month after hardening of the seedlings, while “Cao San”, “Dok” and “VASI-5” had not produced any flowers yet. During the second week of the flowering period the rest of the plants of “RZ Adona” and “1507” had already started flowering, followed by “Cao San” (90%), “Dok” (60%) and “VASI-5” (50%). Some plants of the Asian genotypes “Dok” and “VASI-5” started flowering rather late, with around 40% and 50% of their plants, respectively, starting the production of flowers in the third week of the flowering period.

In the summer of 2002 (Fig. 5-18), again some plants (clones) of Asiatic genotypes “Dok” and “VASI-5” flowered rather late; they produced the first flower about 5 to 6 weeks after transplantation.

Meanwhile all the clones of genotypes “RZ Adona” and “1507”, as well as most of the clones of “Cao San” flowered within the first month after transplanting the seedlings in the greenhouse.

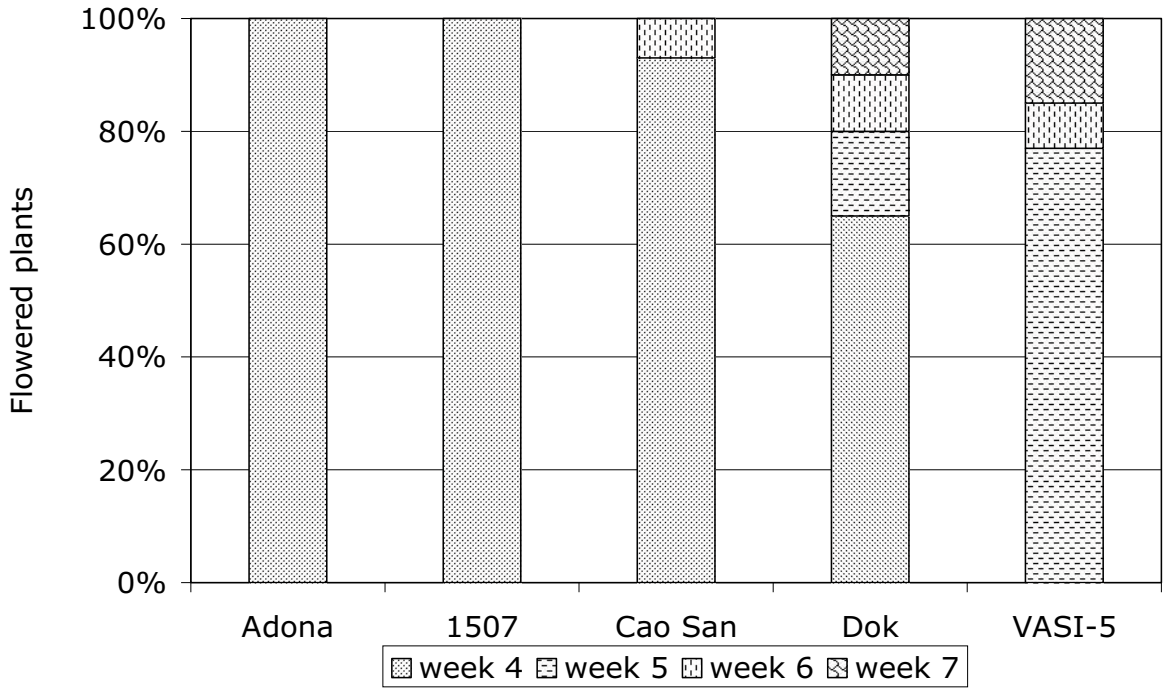


Fig. 5-18 Beginning of eggplant's flowering period in a greenhouse summer cultivation

It was noticeable, already from the third experimental phase during the summer of 2003 (Fig. 5-19) all clones of Asian origin started flowering at the same time and had the most intense period of flower production in June similar to that of "RZ Adona". This result indicates that the investigated Asian genotypes were a good starting plant material for the selection purposes of this study. Therefore, clones could be selected that flower in early summer, a characteristic that makes them appropriate for commercial greenhouse cultivation in temperate regions.

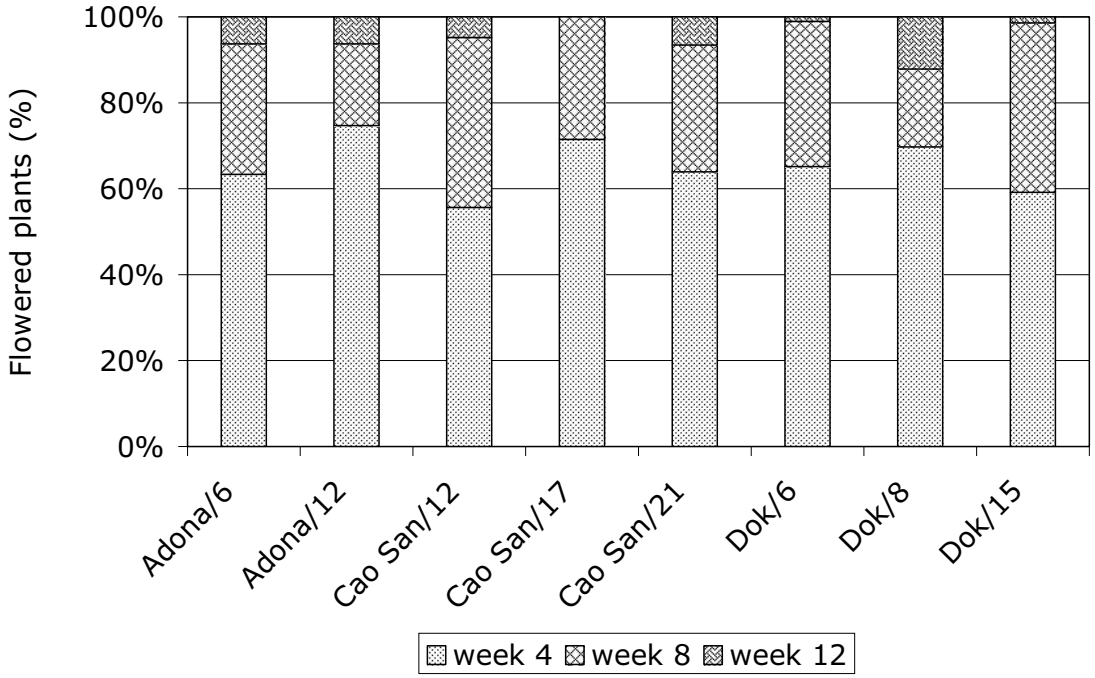


Fig. 5-19 Beginning of the flowering period of the eggplant clones in a greenhouse summer cultivation

Discussion

The most striking differences among the investigated eggplant genotypes were those regarding their generative development. They gave strong evidence of seasonal dependency for their generative growth; even though there was indication of the influence of the genotype on the floral characteristics of the eggplant genotypes as well. The eggplant flowered at higher light radiation level with the subsequent increase in temperature that it represents. Eggplant is considered a day neutral crop (Prinz, 1989; Sutarno *et al*., 1993). Consequently, it generates flowers regardless of photoperiod if temperatures are appropriate (Serçe and Hancock, 2005). The results of this study are in agreement with this statement because the investigated eggplant genotypes flowered both in winter and summer cultivations. During the investigation the day and night temperatures oscillated in the greenhouse within the tolerance limit of aubergine. In winter, artificial light was provided to ensure a minimum of 10 Klx light intensity inside the experimental area (Fig. 4-2, Fig. 4-3). Nevertheless, there were differences regarding the number of days to flowering per genotype. The plants of the high yield variety "RZ Adona" that served as control in the experiments flowered among the first in both winter and summer (see Fig. 5-17, Fig. 5-18). The same applies to the clones obtained from that cultivar that were cultivated in the summers of 2002 (see Fig. 5-19) and 2003.

The plants of Asian origin produced flowers in both seasons as well, just that some Asian plants did so earlier than the others, regardless of the time of the year. This finding suggests a

stronger influence of the genotype on the beginning of the flowering period than season to season climate changes. Alburquerque *et al*. (2004) studied the floral characteristics of horticultural species by investigating flower production, flower quality and final fruit set of nine cultivars in three consecutive years. They concluded that floral biology seemed to be more influenced by a genetic component that year to year weather variability.

- Production of flowers and fruit set

In this study, the total number of flowers produced by every plant of each genotype (Fig. 5-20, Fig. 5-21) and clone (Fig. 5-22, Fig. 5-23) was registered, as well as the total number of harvested fruits. Fruit set was calculated as the percentage of fruits per total flower number. The differences of the generative development were statistically proven in the first experimental phase of this study (Fig. 5-20).

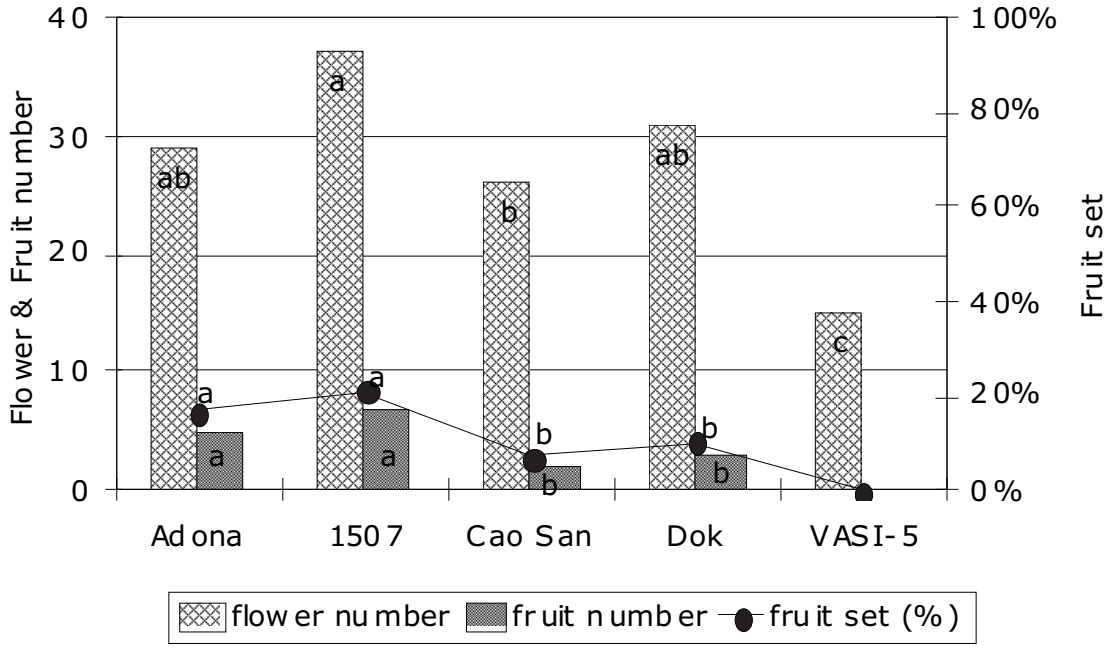


Fig. 5-20 Eggplant's generative development in a greenhouse winter cultivation (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

In the winter of 2001-02 (Fig. 5-20) the control "RZ Adona" and the Asian genotype "1507" obtained the highest results of the total flower and fruit number as well as the fruit set percentage, compared to that of "Cao San", "Dok" and "VASI-5". "RZ Adona" produced 5 fruits out 29 flowers for a fruit set of 17 %. Similarly, "1507" obtained 7 fruits from 37 flowers for a fruit set of 21 %. The total number of flowers produced by "Cao San" and "Dok" was 26 and 31, respectively. The number of fruits obtained from "Cao San" (2) and "Dok" (3) corresponded to a fruit set percentage of 7 and 10, respectively. "VASI-5", despite that produced flowers (15), did not produce fruits at all.

In the summer of 2002 (Fig. 5-21) the flower number and the percentages of fruit set increased for all genotypes, suggesting a seasonal effect on both the flower production and the character conversion of flowers to fruits for this solanaceous crop.

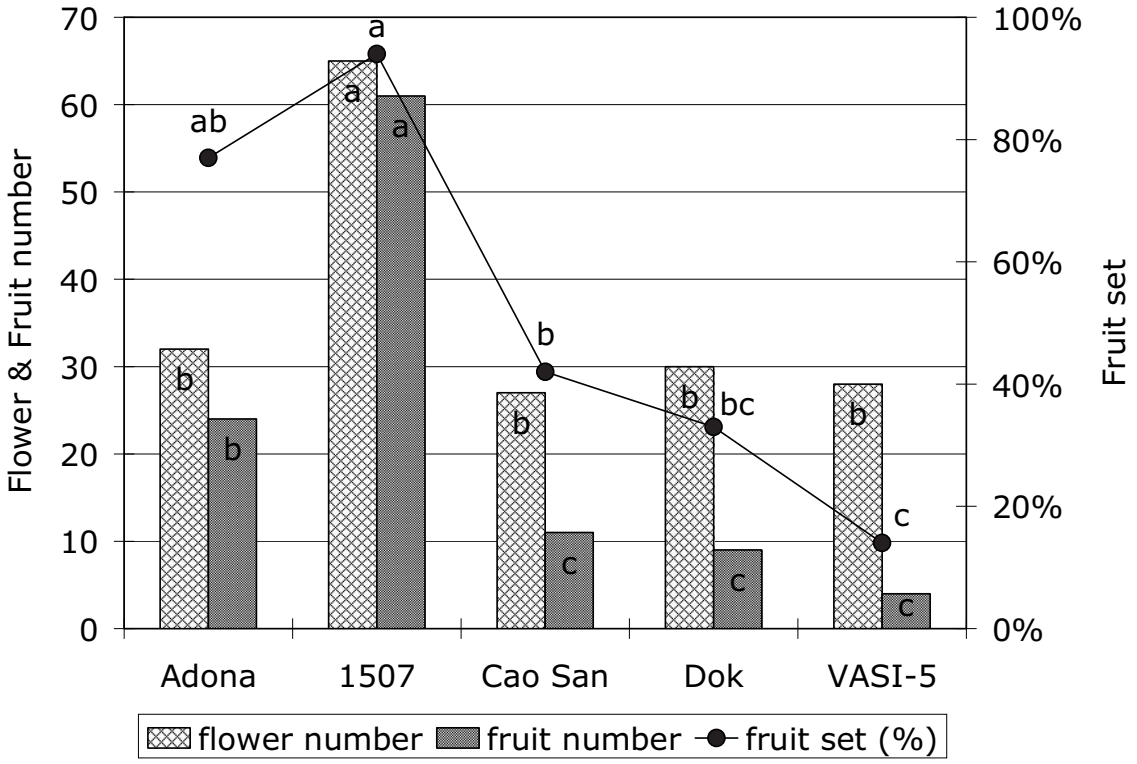


Fig. 5-21 Generative growth of "RZ Adona" and four Asian eggplant genotypes in a greenhouse summer cultivation (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

Asian eggplant genotype "1507" had the highest number of both flowers (65) and fruits (61) for the largest fruit set percentage (94) of the season. Statistically, "RZ Adona" (32) obtained similar flower number like the "Cao San" (26), "Dok" (30) and "VASI-5" (28). The fruit number, nevertheless, was different between "RZ Adona" (24) and "Cao San" (11), "Dok" (9) and "VASI-5" (4). The fruit set percentage gave intermediate values for "RZ Adona" (77), "Cao San" (42) and "Dok" (33); while "VASI-5" (14) obtained the lowest value of this parameter (Fig. 5-21).

In the summers of 2002 (Fig. 5-22) and 2003 (Fig. 5-23) clones were chosen from all genotypes and analysed regarding their generative development. The differences of the generative development among clones were not always statistically different, result that may be attributed to the high variability of the results or to the fact that, in some cases, there were just 2 replicas available per clone.

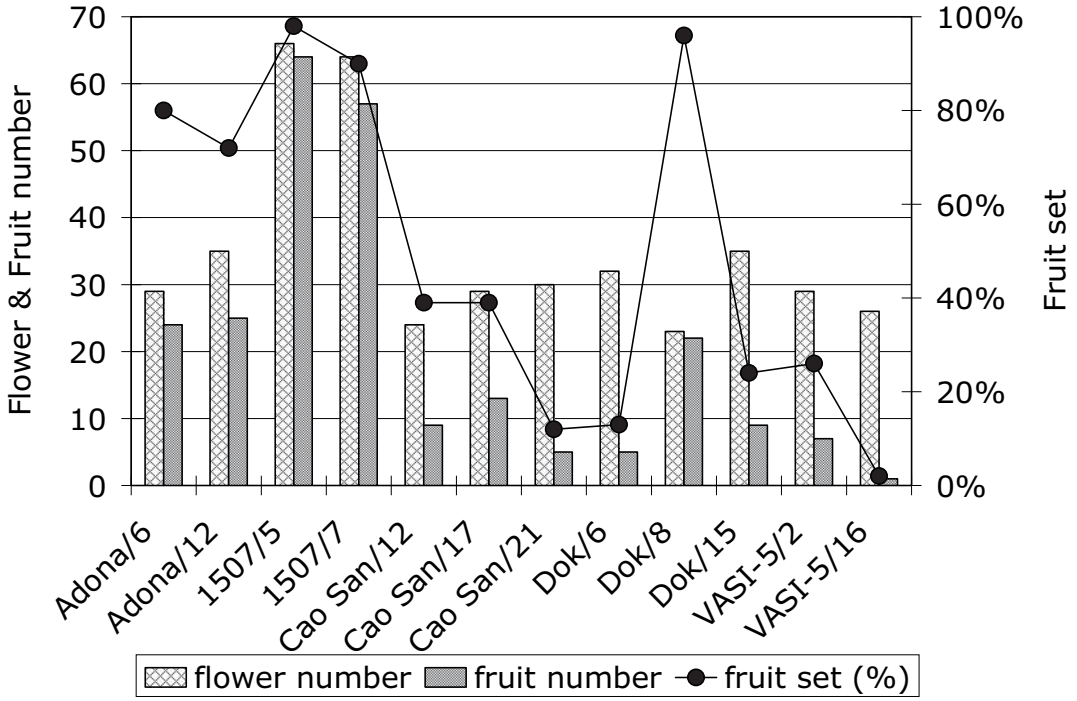


Fig. 5-22 Generative development of the eggplant clones in a greenhouse summer cultivation

In the summer of 2002 the highest results of both, the flower (66, 64) and fruit number (64, 57) as well as the fruit set percentage (98, 90) were those of clones 5 and 7, respectively, of Asian genotype "1507". The rest of the clones of Asian origin had similar flower number among themselves as well as between them and that of the clones of the control "RZ Adona". The results ranged between 22 ("Cao San"/6) and 35 ("RZ Adona"/12) flowers per plant for the whole flowering period. The production of fruits and the fruit set percentage as well were most variable among the clones obtained from the genotypes "Cao San", "Dok" and "VASI-5" of Asian origin. Despite that the production of fruits varied from 0 to 9 (7 clones of Asian origin), between 13 and 16 (3 clones of Asian origin) and from 22 to 25 (1 clone of Asian origin and the 2 clones of the control "RZ Adona") no statistical differences were found for this parameter among those results, which may be due to the variability of this character. The fruit set percentage of the clones "Dok"/8 (96), "Adona"/6 (80) and "Adona"/12 (72) were higher than that of the other Asian clones whose values ranged between 0 ("Dok"/21) and 39 (clones 2 and 17 of "Cao San"). Meanwhile, "Cao San"/6 obtained a fruit conversion rate of 64 % (Fig. 5-22).

The selection of the clones for the last experimental phase was done upon their generative development. In the summer of 2003, the parameters flower number and fruit set brought no statistical differences among clones (Fig. 5-23).

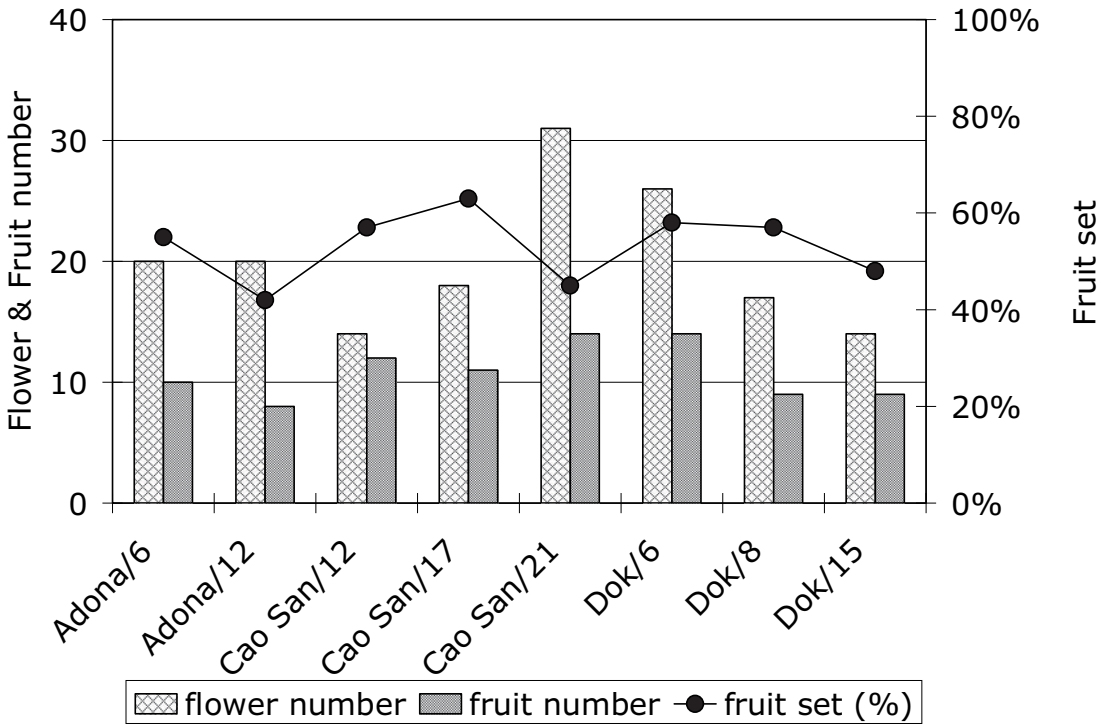


Fig. 5-23 Generative growth of the new selected eggplant clones in a summer cultivation under greenhouse conditions

The flower number ranged from 14 to 31, the fruit number varied between 8 and 14 and the fruit set percentage fluctuated between 42 and 63. Remarkably is this ratio of clone "Cao San"/21 that produced in average 31 flowers per plant had a fruit set percentage of 45; while clone "Cao San"/17 that obtained 18 flowers per plant had a fruit set percentage of 63 (Fig. 5-23). In the summer 2005 the cultivar "RZ Ritmo" was the control in the experiments, it is also a F_1 hybrid of the Rijk Zwaan breeding company, like "RZ Adona". The number of flowers and fruits, generally, decreased for all genotypes in this growing season. Nevertheless, the conversion rate of flowers into fruits was around 40 %, which increased effectiveness and ensured a good harvest. The obtained results regarding the generative development of eggplant indicate a seasonal influence on the development of this crop.

Discussion

Both, the control "RZ Adona" and the Asian genotypes, generated more flowers and fruits in summer than in winter cultivation (see Fig. 5-20, Fig. 5-21, Fig. 5-22, Fig. 5-23. This result is not that surprising considering the ecology of the eggplant. The interesting finding has to do with the fact, that striking differences were found among the clones obtained from the Asiatic eggplant genotypes regarding their response to similar environmental conditions. The latest suggests a genetic component on the generative development of these individuals, and may be useful when selecting parents for breeding programmes or new genotypes for protected

cultivation of eggplant. Different authors report about the influence of climatic conditions and genotypic dependence on the production of flowers and yield of many species. As read in Cemek *et al*. (2005), temperature has a considerable influence on crop timing and yield (Pearson, 1992). According to Alburquerque *et al*. (2004), many studies on different fruit varieties report the influence of climate on pollination, stigma receptivity, ovule fertility and longevity as well as fruit set (Burgos *et al*., 1993; Guerriero *et al*., 1985; Furukawa and Bukovac, 1989). However, it is postulated that the flower buds produced is among the factors influencing productivity. This factor was found to be genotype dependent (Bellini and Gianelli, 1975), and high variability in bud density (Okie and Werner, 1996) was reported in other horticultural species. The transformation from flower to fruit occurs after anthesis. Therefore, flower bud drop has a negative influence on fruit production and yield (Alburquerque *et al*., 2004). Even though, the reasons for the drop of flowers are uncertain, it is related to improved fruit set (Jackson and Hamer, 1980). In many species the drop of flowers is a normal process (Guitián, 1993) and the quality of a flower or the capacity of a flower to become a fruit (Williams, 1965) differs among flowers (Guitián, 1994). There are many factors affecting flower quality, including genetic and morphological characteristics (Louri *et al*., 1996), nutritive status (Lloyd, 1980) or reserves of starch (Rodrigo *et al*., 2000). Abiotic factors like water stress, chilling and high temperature seem to affect flower drop as well (Alburquerque *et al*., 2004, Weinberger, 1956; Brown, 1958; Monet and Bastard, 1971), as well as flower bud initiation and development (Caprio and Quamme, 1998).

5.3.4 Morphological characteristics of the fruits and yield of eggplant in the greenhouse

- Morphology of the fruits of the investigated genotypes

Particularly variable were both the fruit number and size produced by the different clones. Eggplant Asian clone “1507”/ 7 averaged the largest fruit number (23), while clone “RZ Adona”/ 12 produced only about 9 fruits; their yield was similar nevertheless, with 2,1 kg each; which indicates that genotypes with smaller fruits may have similar yield in kg to “RZ Adona” because the fruit number they produce is higher. The latest findings add more weight to the hypothesis that the Asian cultivars used in our study, were a good starting plant material for the selection objectives of this research (Table 5-7).

Table 5-7 Fruit characteristics of the eggplant cultivars (IBPGR, 1998; Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

Genotypes	n	fruit weight (kg)	fruit size (cm)			
			length	IBPGR	breadth	IBPGR
"RZ Adona"	51	284.8a	14.6a	long	7.4a	large
"1507"	78	87.0b	14.6a	long	3.6c	intermediate
"Cao San"	117	58.3c	10.2b	long	3.6c	intermediate
"Dok"	150	60.7c	10.3b	long	4.7b	large
"VASI-5"	93	55.3c	10.0b	long	3.8c	intermediate

Discussion

The variability of eggplant's fruits is known to be very high (Prinz, 1989; Lal, 1991; Sutarno *et al*., 1993). Similarly, the fruits of the eggplant genotypes of Asian origin (Fig. 5-) involved in this study showed a variety of colours and shapes (see also Fig. 5-16). Those phenotypical differences made difficult to determine the right harvesting time for the fruits of the Asian plants. Within the Asian genotypes, the size of the fruits seemed to be more affected by genetic variability, result that is similar to those found by Lal, (1991).

Fig. 5-24 Fruits from Asian eggplants "Dok" and "1507" vary in shape and colour

- Yield in the forms of fruit load per plant and fresh fruit weight

The yield per plant is described in the forms of fruit number and fruit load in kg of the harvested fruits per genotype in winter (Fig. 5-25) and summer (Fig. 5-26) in the greenhouse. Clones (Fig. 5-27, Fig. 5-28, Fig. 5-29 and Fig. 5-30) from those genotypes were tested in summer. The statistically different results of this important agronomic parameter supports the hypothesis that it is possible the selection of new eggplant genotypes suitable for greenhouse cultivation from aubergine varieties. The results are averages of one winter and four summer cultivation seasons in the greenhouse.

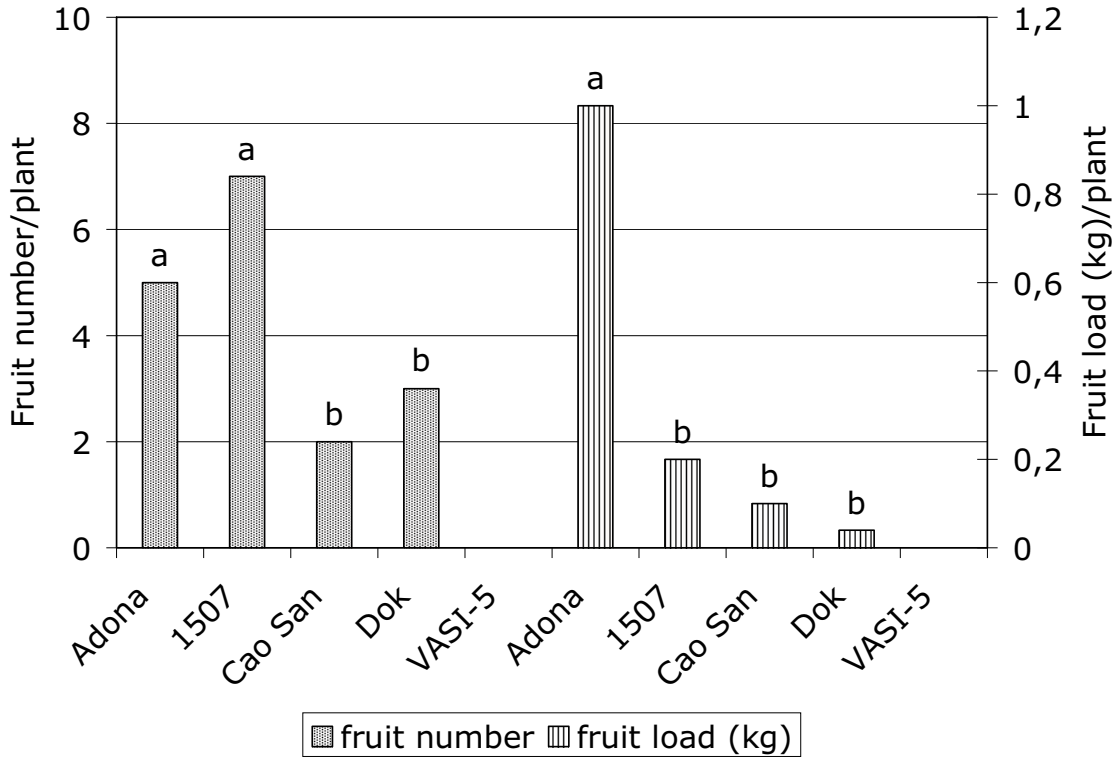


Fig. 5-25 Yield of five eggplant genotypes per plant in a greenhouse winter cultivation (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

The plants of "RZ Adona" produced dark purple, elongated fruits (see Fig. 2-5). Among the plants of Asian origin the fruit colour had varied from white till lilac, some fruits being striped as well. Besides, the shape of the berries was diverse, too. Thus, high genetic variability of the fruit colour and shape was observed in the Asian eggplant genotypes within cultivars. The fruits of "1507", "Cao San", "Dok" and "VASI-5" had a diversity of colours ranging from lilac with stripes to purple black, being yellow and white as well. The size and shape were very diverse, too (see Fig. 5-16, Fig. 5-24). Despite that in the winter experiment (Fig. 5-25) each plant of all genotypes developed flowers not all of them formed fruits. "RZ Adona" and "1507" yielded more fruits than the other genotypes. "Cao San" and "Dok" produced some fruits in winter, while "VASI-5" did not produce fruits at all.

Concerning the fruit number produced by each clone, a result similar to that related in the literature was obtained. It is those genotypes producing smaller fruits that produced the biggest number of them; therefore, "Dok"/6 and clones 12 and 21 of "Cao San" exhibited the highest results for this trait. "RZ Adona" produced fruits with more than 200 g of weight each at harvesting time, and gave the best yield of the season, followed by "1507", "Cao San" and "Dok" (Fig. 5-25).

The same eggplant genotypes were evaluated in summer cultivation (Fig. 5-26) under controlled conditions.

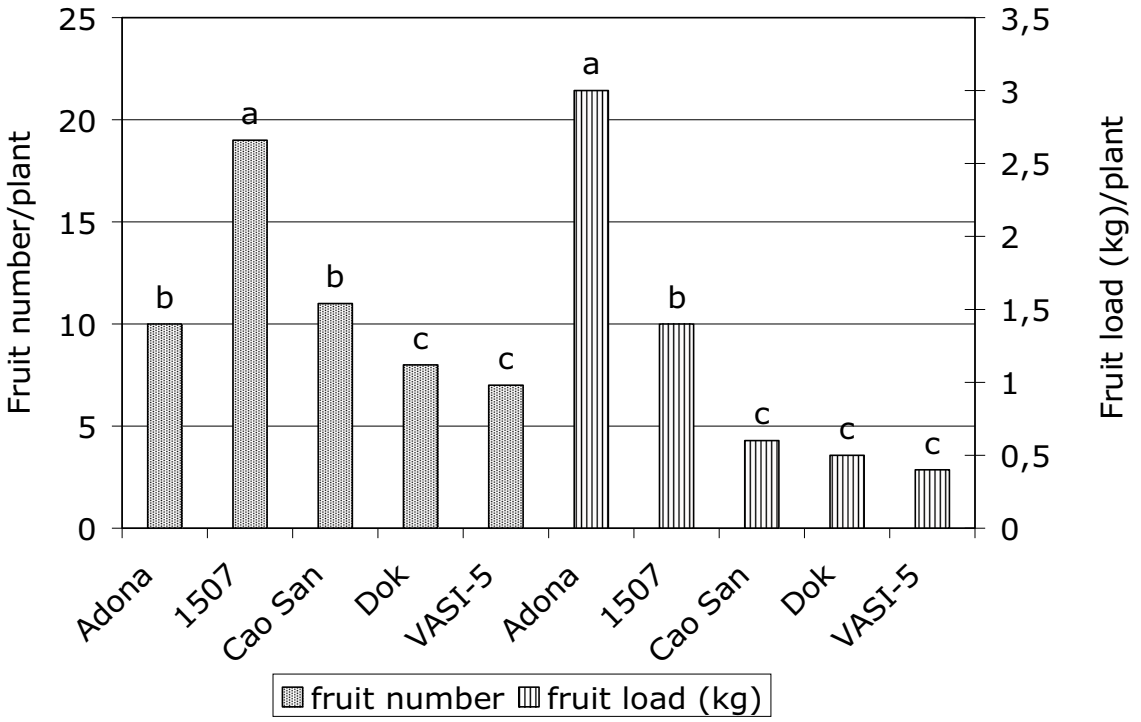


Fig. 5-26 Yield of five eggplant genotypes per plant in a greenhouse summer cultivation (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

In the summer experiments "RZ Adona" had the biggest fruits, but "1507" produced more fruits than the others, and its yield in kg was second biggest after that of "RZ Adona". Statistically, "Cao San", "Dok" and "VASI-5" had a similar harvest (Fig. 5-26).

In 2002 from the genotypes "RZ Adona", "1507", "Cao San", "Dok" and "VASI-5" clones were selected and compared according to their average yield.

The characteristics of the yield among different clones are described during the summer experiments of 2002 (Fig. 5-27) and 2003 (Fig. 5-28). "RZ Adona" and "1507" only had 2 clones with at least 2 plants each. The other genotypes had between 6 and 9 clones with a replica available.

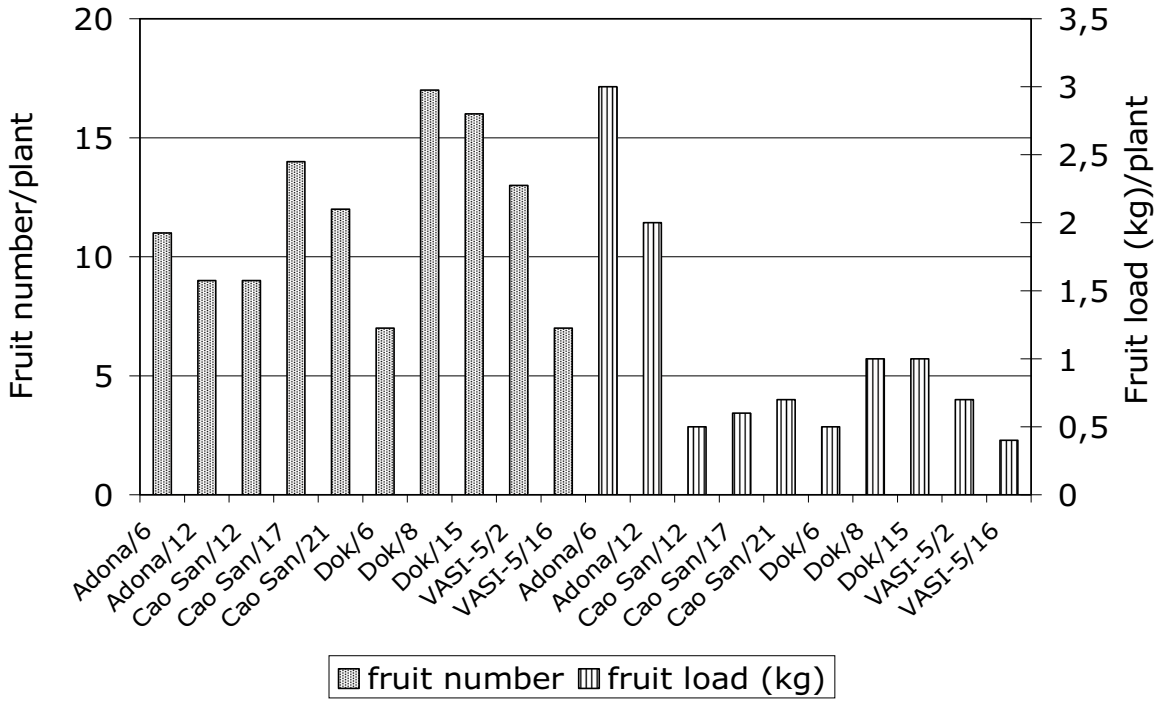


Fig. 5-27 Yield of eggplant clones under controlled production conditions in summer 2002 (ANOVA, $P<0.05$)

"RZ Adona" had the highest yield in kg (3,4) and Asian genotype "1507" produced the largest fruit number (23). "Dok" exhibited the highest variability: its clones 8 and 15 yielded more than 16 fruits each with 1 kg in total, compared to up to 9 fruits and a half a kg for the rest of its clones. The clones of genotype "VASI-5" had the lowest yield compared to that of other clones. That result is comparable to the one obtained in the first experimental phase (Fig. 5-27).

The yield of the clones in summer 2003 is represented in Fig. 5-28.

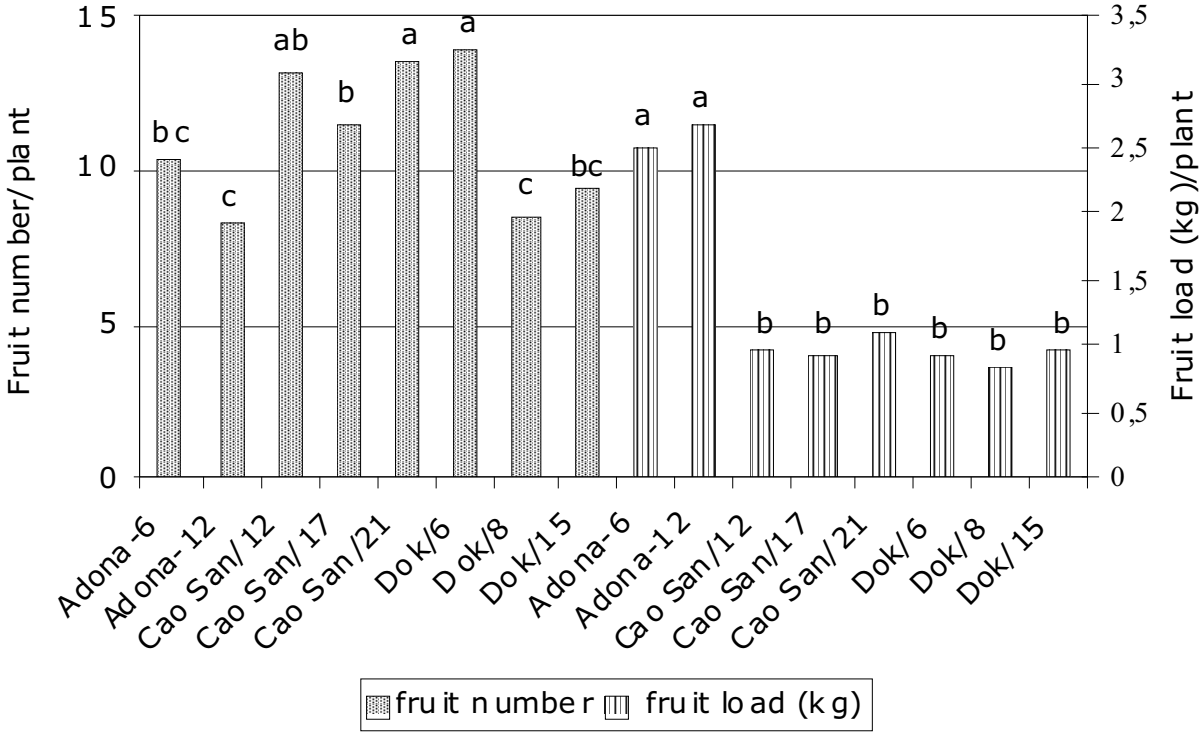


Fig. 5-28 Yield of eggplant clones under controlled production conditions in summer 2003 (Tukey HSD & Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

Clones 6 and 12 of "RZ Adona" produced the highest yield, followed by "Cao San"/21, "Cao San" /12 and "Dok"/15. Clones "Cao San"/17, and clones 6 and 8 of "Dok", in this order, gave the least harvest. These results were statistically different (Fig. 5-28).

The high yield variety "RZ Ritmo" served as control in the experiments from summer 2004 (Fig. 5-29) and 2005 (Fig. 5-30). In these trials, the eggplant clones (10 plants each) were planted in peat slabs as in regular commercial cultivation.

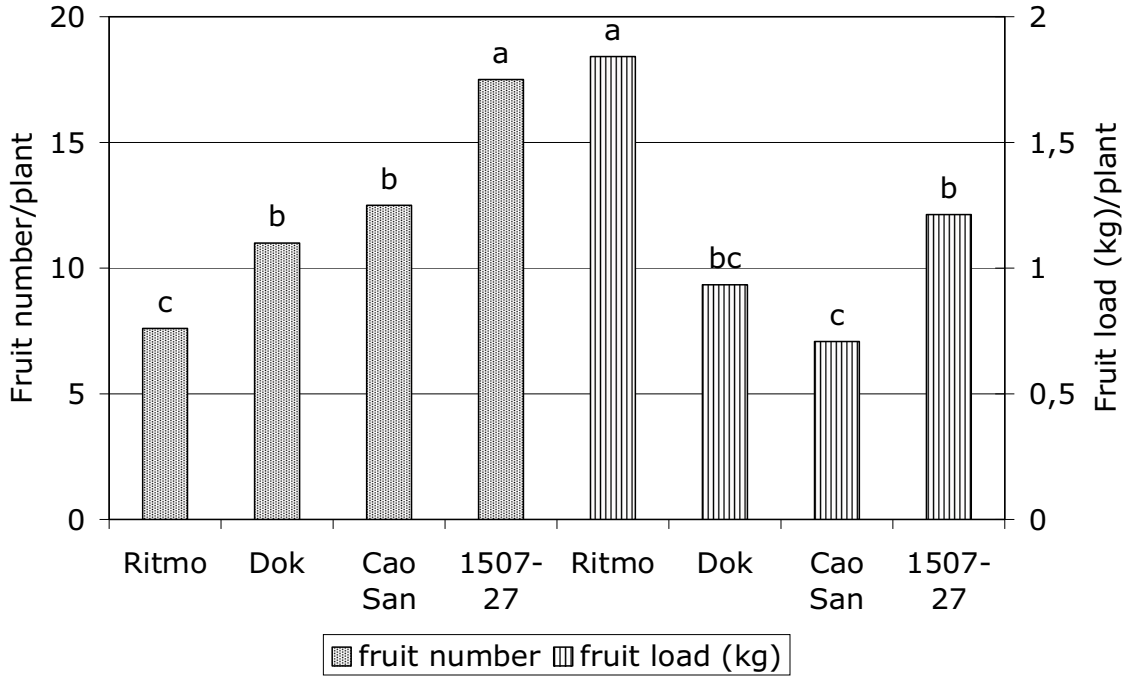


Fig. 5-29 Yield of eggplant clones per plant under commercial cultivation conditions (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

In the summer of 2004, "RZ Ritmo" produced its typical purple black berries with a fruit load of 1.5 kg per plant for best results, statistically proven, of this parameter; followed by Asian clone "1507"-27 with a yield per plant of 1.2 kg. The Asian clones "Dok" and "Cao San" yielded each about 1 and half a kg of eggfruits per plant, respectively. Once more, Asian clone "1507"-27 (17,5) produced the highest number of fruits, followed by "Cao San" (12,5), "Dok" (11) and "RZ Ritmo" with 7,6 fruits per plant (Fig. 5-29).

Usually, the eggplant clones were propagated by seed, only in 2005 (Fig. 5-30), plantlets of different origin (from seed –s-, or micropropagated –i.v.-) were used to evaluate their genetic stability in a commercial-like cultivation system.

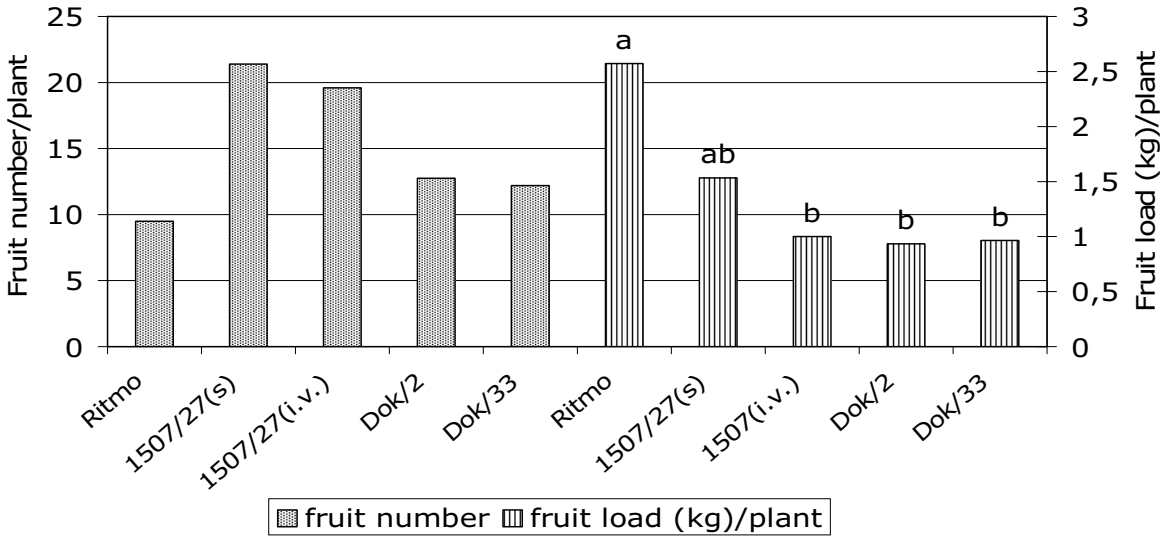


Fig. 5-30 Yield of eggplants clones from seeds or *in vitro* plantlets under commercial cultivation conditions (Tukey HSD& Kruskal-Wallis P< 0.05, different letters stand for statistical differences)

In the summer of 2005 (Fig. 5-30) were obtained similar results on the eggplant yield like in 2004. Therefore, "RZ Ritmo" with more than 2 kg had the highest yield, and the Asian clones produced between 1 and 1.5 kg of eggfruits per plant. The fruit number of the eggplant clones was very variable (from 10 up to 21), therefore, no statistical differences could be established. Nevertheless, the clones showed a pattern in this character that is similar to the one in previous experiments. Thus, Asian clone "1507"-27 (21), whether propagated from seeds or from *in vitro* plants, produced more fruits than the other Asian clones and the control "RZ Ritmo".

Discussion

In this investigation, the cultivar "RZ Adona" and three Asian eggplant genotypes produced fruits in winter and summer cultivation. Only, Asian eggplant cultivar "VASI-5" did not set fruits in winter (see Fig. 5-20). Also, the summer production of fruits was higher for all investigated genotypes, than in winter (see Fig. 5-20, Fig. 5-21, Fig. 5-22, Fig. 5-23). This result points at a general effect of the climate on eggplant productivity (Arias *et al*., 2003). It is known that eggplant is a warm season crop (Prinz, 1989; Paksoy and Akilli, 1994; Rindels, 1997; Chen *et al*., 2001) and that protected cultivation makes it possible to obtain increased crop productivity, by maintaining a favourable environment for plants (Cemek *et al*., 2005). In this study a glass greenhouse served as experimental area. It should be noticed, however, that in summer, season that favoured the productivity of this crop, different yields per plant were obtained from the Asian eggplant clones, despite the controlled climatic condition in the growing area. This result suggests that these plants followed a pattern of fruit set independent

of the weather conditions. In favour of this finding it is reported that the influence of the genotype was more determinant than the seasonal effect on fruit yield, and according to Aktinson and Taylor (1994) and Aktinson and Lucas (1996) the fruit set, which is in direct relation to the yield, is at least partially genotype dependent in other horticultural species, as read in Alburquerque *et al*. (2004).

6 Conclusions and Recommendations

Background

Human and natural selection has lad to a wide genetic diversity among cultivated and wild species of eggplants. It is often reported that while the cultivated varieties of aubergine are susceptible to various pests and diseases, as well as to abiotic stress conditions, the contrary occurs with the wild genotypes, which are resistant to most of the pathogens and abiotic factors that are detrimental to the development of this crop. Therefore, genotypes and landraces obtained by natural selection or human breeding that grow in eggplant's centres of origin, seem to be an interesting source of desired agronomical traits to be regained into the cultivated species (Collonnier *et al*., 2001; Kashyap *et al*., 2003; Sękara *et al*., 2007).

6.1 Summary of the main results

In this research, were studied eggplant genotypes of South East Asian origin and compared to European bred varieties for intensive crop cultivation. The Asian eggplants were screened under protected conditions in order to obtain new stable genotypes for greenhouse cultivation systems. Selected Asian eggplants showed early flowering characteristic (Fig. 5-19), which is very important for greenhouse cultivation, as well as similar yield (Fig. 5-25, Fig. 5-26, Fig. 5-27, Fig. 5-28) to the control European varieties. Besides, it was possible to cultivate the selected Asian eggplants under commercial-like conditions in the greenhouse without affecting their yield (Fig. 5-29, Fig. 5-30). Thus, looking carefully into the genetic resources available for cultivated crops existing in their centres of origin, it is possible to find those interesting characteristics from agronomical and economical viewpoints, so as to decrease costs in the selection process, as long as biodiversity is preserved. Based upon those results it is recommended the introduction of new eggplants genotypes of Asian origin in the European markets, and for selection purposes. Thereby, the findings of this study related to **Chapter 3. Aim of the research** are described based on **General and specific objectives** (Subchapters 3.1. and 3.2.) and on the **Research questions** (Subchapter 3.4.)

Research Question 1: How the Asian eggplant genotypes respond to tissue culture techniques?

The use of biotechnological methods may speed up the selection process. Usually, micropropagation, as well as other tissue and organ culture techniques, is used for research purposes on eggplant, which is propagated by seeds. In this study, propagation *in vitro* or micropropagation of eggplant (Subchapter 5.1.2.) was used as a tool to speed up the multiplication of clones. The clones of Asian eggplant genotypes were obtained from *in vitro*

cultures then acclimatised and characterised in the greenhouse, where the plantlets developed to normal plants that reached full maturity, including a good yield (Fig. 5-30) comparable with that of the controls, the typical 'European' varieties "RZ Adona" and "RZ Ritmo".

The positive response of eggplant in general, and of the Asian eggplant genotypes, specifically, to *in vitro* multiplication (Subchapter 5.1.2), made it possible to propagate and obtain available plant material for the purposes of this research. The response of the Asian eggplant genotypes object of this study, to callogenesis and organogenesis, is described in Subchapter 5.1.3. The results of these experiments were partly in agreement with those described in the literature and need further investigation.

Research Question 2: Is eggplant's phenotypical diversity linked to a genetic marker?

The preliminary results of the study using the RAPD genetic marker technique are exposed in Subchapter 5.2. This experiment was carried out to link or explain the morphological diversity of the Asian eggplant genotypes at molecular level. Firstly, DNA isolation by Quiagen-Plant-Kit method allowed obtaining good quality DNA from the Asian eggplant plantlets. It followed the adjustment of the PCR reaction conditions, whose produce showed the genetic pattern of the studied eggplant clones when exposed to different primer combinations. The plant material used to obtain DNA used as template in the PCR reaction, were eggplant clones from Albania, Europe and South East Asia. The findings on this preliminary genetic study did not bring out striking genetic differences among those eggplant genotypes. Similarly, Collonnier *et al*. (2001) reported scarce genetic diversity of the eggplant genome, independently of the geographical origin of the species. Further experimentation is needed to find genetic markers in eggplant that can be linked to external or physiological characteristics in order to facilitate the selection and genetic breeding of the crop.

Research question 3: Have organic or inorganic substrates for greenhouse cultivation an influence on the growth of the Asian eggplant genotypes?

In Subchapter 5.3.1. is depicted the development of the Asian eggplant genotypes in 2 different substrates, one organic (B_{700}) and the other one inorganic (Perlite). In both cases, the results on the vegetative growth of the crop were compared to that of the high yielding variety "RZ Adona". The trial took place in winter where artificial light was supplemented when less than 10 Klx were measured outside the experimental area, and day as well as night temperature was kept within the toleration limits for aubergines. In this study, both the Asian eggplant genotypes and the high yield variety "RZ Adona" that were tried in organic substrate grew at a faster pace than those growing in perlite. One month after transplanting the young plants to each substrate, the ones in perlite reached about half of the vegetative development compared to the ones in organic substrate. At this point, the experiment with the plantlets

growing in perlite was stopped. The trial continued for the plantlets growing in organic substrate until the harvest time was reached by the adult plants.

Research question 4: How are the vegetative and generative developments of the Asian eggplant genotypes under greenhouse conditions?

The Asian eggplants genotypes developed into normal adult plants in the greenhouse (Subchapter 5.3.2.). The morphological (plant height; number and shape of leaves; weight, colour and shape of fruits) and physiological (beginning of flowering; production of flowers; fruit conversion rate) differences among and within the genotypes of Asian origin was evident during the whole developmental cycle. The first two experimental phases served as to screen the plant material of Asian origin for pre-selection of clones, in order to obtain a uniform plantation, based upon the phenotypic characteristics. Besides, the acclimatisation and selection processes of the Asian eggplant genotypes for greenhouse cultivation focused on the eggplant clones of Asian origin that flowered early, too. The last three experimental phases of the whole study, gave insights on the suitability of the new selected Asian eggplant clones for cultivation under greenhouse conditions. The new selected clones of Asian origin flowered rather early and had the highest flowering period at the same time. Thus, commercial cultivation in the greenhouse of such Asian eggplant genotypes can be achieved. Specifically for the last experimental phase, those clones were tried in a commercial like cultivation system with peat slabs as a substrate and drip irrigation system. Also, plantlets cloned *in vitro*, as well as seeds from mature fruits of the previous season were successfully used as propagation material for the new selected Asian clones of eggplant.

Research question 5: Is there a relationship between the beginning of the flowering period of the Asian eggplant genotypes and the yield?

Under Subchapter 5.3.3 Generative development of the eggplant genotypes it described the beginning of the flowering period that affected the production of flowers and the yield of the Asian eggplant genotypes, too. Those results clearly showed that the Asian eggplant genotypes flowering in a similar period like the typical 'European' varieties "RZ Adona" and "RZ Ritmo" had a comparable yield to them, too. The results of this parameter indicated genotypic dependence in the flowering pattern of the Asian eggplant, even though the influence of the climatic conditions seemed decisive as well. It was observed that when temperatures were around 25$\pm$2°C together with approximately 70% relative humidity in the experimental area, the plants produced many flowers, and quite a few dropped or aborted. It was noticed that more direct impact on the yield had the ability by the plants to convert flowers into fruits or the fruit conversion rate, than the number of flowers produced per plant.

Research questions 6 and 7: Is it possible to obtain stable eggplant genotypes for greenhouse cultivation system by screening eggplant landraces of South-Asian origin? Would the new selected Asian eggplant genotypes strive for similar harvest like the high yield varieties "RZ Adona" and "RZ Ritmo", in a commercial-like, 'substrate culture' cultivation system in greenhouse?

In this research, Asian eggplant genotypes obtained by local breeders in Vietnam were compared with the typical 'European' varieties "RZ Adona" and "RZ Ritmo". The vegetative and generative developments as well as the yield of each genotype were monitored in the greenhouse. Clones were obtained from the Asian group of plants that exhibited differences in their vegetative development and morphological characteristics, had comparable yield to that of the high yield variety "RZ Adona" and "RZ Ritmo", and tended to flower early. The latest is a highly desirable feature in temperate regions, where light is a constraint for the production of vegetables in the greenhouse. The selection strategy probed to be suitable for obtaining new eggplant genotypes of Asian origin able to grow and strive under greenhouse conditions in temperate regions. Additionally, the cultivation of this crop under greenhouse conditions will contribute to obtain good quality fruits and to avoid the negative influence of adverse weather or abiotic stress conditions. The latest is especially true for western countries with a tendency for the consumption of new eggplant types with different taste and colour, where high quality of such fruits is compulsory. Besides, the resident Asian communities will also appreciate the availability in the western markets of their traditional vegetables.

6.2 Conclusions

- Micropropagation and *in vitro* manipulation of eggplant

1. The propagation *in vitro* of the eggplant genotypes permitted to obtain sufficient plant material for the purposes of the research.

2. *In vitro* cloning of eggplant from nodal segment explants was achieved without adding PGR in the culture medium.

3. The genotype of eggplant did not seem to have influenced the response of this crop to micropropagation.

4. The eggplant genotypes of Asian origin were more susceptible to *in vitro* contamination than the Dutch variety "RZ Adona".

5. Callogenesis and organogenesis of Asian eggplant genotypes and "RZ Adona" was induced with 2 PGR at distinct concentrations, on nodal segments as well as on cotyledons explants.

6. The origin of the explant, e.g. nodal segment or hypocotyls, cotyledon, and the PGR and its concentration as well, played a key role in the eggplant response to callogenesis and

organogenesis.

7. Indirect regeneration of eggplant shoots was more efficient from cotyledon than from nodal segment explants.

- Genetic study of Asian eggplant genotypes

1. RAPD-PCR gave insights on potential genetic differences in Asian eggplant genotypes.

2. The eggplant genome seemed to be similar, independently of the geographical origin of the crop.

3. 3 out 11 random genetic markers depicted different pattern on the eggplant genome.

- Cultivation of eggplant in the greenhouse

1. Eggplant developed well in both winter and summer cultivation in the greenhouse, but in winter artificial light is necessary. Nevertheless, the vegetative and generative growths were better in summer than in winter.

2. The organic substrate favoured better growth of eggplant in the greenhouse.

3. Eggplant, specially the genotypes of Asian origin, seemed to depend on the climatic conditions, e.g. irradiation, temperature, to develop their genetic potential.

4. The differences in the vegetative and generative development, as well as in the yield between the 4 eggplant genotypes of common Asian origin allowed the selection of Asian clones with early flowering behaviour.

5. The Asian clones of eggplant "1507" that flowered in early spring, also obtained yields comparable to that of the hybrid varieties "RZ Adona" and "RZ Ritmo".

6. The Asian eggplant genotypes produced smaller fruits but in higher number than "RZ Adona" and "RZ Ritmo".

7. The origin of the plant material for propagation of eggplant, e.g. seeds, *in vitro* plantlets, did not seem to have influenced the development and fruit production of this crop.

8. The selected Asian clones had a stable yield under a commercial-like cultivation system.

6.3 Recommendations

- *In vitro* manipulation of eggplant

1. Further investigation is needed in order to assess the influence of PGR on the response of the Asian eggplant genotypes to callogenesis, organogenesis and somatic embryogenesis.

2. Indirect plant regeneration of the Asian eggplant genotypes shall be revised aiming at increasing the regeneration percentage.

3. Cytological analysis of the Asian eggplant genotypes is necessary to detect vitrification susceptibility.

- Genetic study of Asian eggplant genotypes

1. A deeper study at molecular level would help characterise the genetic pattern of the Asian eggplant genotypes
2. Obtaining genetic markers of the Asian eggplant genotypes would allow a more precise selection process.
3. Increasing the genetic information about the Asian eggplant genotypes would improve systematic classification of this species.

- Cultivation of eggplant in the greenhouse

1. Describe flowering and pollination behaviour of the Asian eggplant genotypes.
2. Characterise the chemical composition of the fruits of the Asian eggplants.
3. Evaluate maturity index of the Asian eggplant genotypes by using non-destructive methods.
4. Evaluation of the acceptance by consumers of the new selected eggplant clones of Asian origin for greenhouse cultivation.

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8 Attachment

Att.I Characteristics of the organic substrate

Description of Organic Substrate B_{700}	
Parameter	Structure
water capacity	65.1%
air capacity	22.2%
pore volume	87.3%
density	122 kg m^{-3}
composition	peat, clay & Cocopor
Other characteristics	
Type of culture	Hydroponic
Container volume	8 L

Att.II Less common eggplant varieties in the USA (Lindgren, 1996)

Cultivar	Description
'While Italian'	Medium-sized white fruit, slightly milder flavor than purple types
'Golden Yellow'	Produces yellow, lemon-sized fruit
'Morden Midget'	Small, bushy plants that bear smooth, medium-sized deep purple
'Applegreen'	Apple green coloured fruit
'White Beauty'	White, medium-sized fruit

Att.III Companies distributors of eggplant seeds all over the world (http://www.sgn.cornell.edu/community/links/seed_companies.html)

Anioleka Seed Company	http://www.seedfest.co.uk
Anseme Srl	http://www.anseme.com
Atlas Seeds, Inc.	http://www.seedquest.com/best/AtlasSeeds/
Bakker Brothers Inc.	http://www.bakkerbrothers.nl
Bioseed Genetics International, Inc.	http://www.premjee.com/bgi.htm
Condor Seed Production, Inc	http://www.condorseed.com
L. Daehnfeldt A/S	http://www.daehnfeldt.com
DeRuiter Seeds	http://www.deruiterseeds.com
East-West Seed International Ltd.	http://www.eastwestseed.com
Genesis Seeds Ltd.	http://www.genesisseeds.com
Golden Valley Seed	http://www.goldenvalleyseed.com
Green-Seeds Co.	http://www.green-seeds.com
Hazera Genetics Ltd.	http://www.hazera.co.il
Indo-American Hybrid Seeds (India) Ltd.	http://www.indamseeds.com
Known-You Seed Company Ltd.	http://www.knownyou.com
Larosa Emanuele Sementi	http://www.larosaemanuele.com
Maharashtra Hybrid Seed Co. Ltd.	http://www.mahyco.com
Nickerson-Zwaan	http://www.nickerson-zwaan.com
Nirit Seeds Ltd.	http://www.niritseeds.com
Seminis	http://www.seminis.com http://www.seminisgarden.com
Shiram Bioseed Genetics India Ltd.	http://www.gp.th.com/bgi.htm
Stokes Seeds Ltd.	http://www.stokesseeds.com
Technisem	http://www.technisem.com
Techspark Seed Co., Ltd.	http://www.techspark.com.cn
Western Seed Espana SA	http://www.western-seed.com

Att.IV Commercial eggplant hybrid varieties in the European market (Anioleka Vegetable Seeds Company, 2003)

Eggplant commercial name	Origin	Description
Italian White	Italy	produces a great abundance of slim white eggplant fruits.
Violetta Lunga	Italy	very productive cultivar with long purple fruits.
Black Beauty	USA	producer of large, nearly pear shaped black eggplant fruits.
Dourga White	France	white eggplant with ivory coloured and thin skin and tender flesh.
Orestiada Greek	Greece	prolific producer of large purple pear shaped eggplant fruits.
Long Oriental Purple (Brinjal)	Pakistan	produces long, tapering Eggplant fruits.
Orestiada Small Greek	Greece	produces small purple eggplant fruits.
Prosperosa	Italy	produces an abundance of round light purple eggplant fruits with white stripes.
Russian Caucasus	Russia	produces long, slim and purple coloured fruits, very thin skinned that contain few seeds and mature in about 80 days.
Kurome Oriental	India	is a long purple fruited variety, heavy early producer of excellent eggplants, fairly thin skinned and rarely bitter.
Lavander Touch	USA	produces long, crescent shaped fruits, the plant produces pastel pink blossoms before setting fruit upon silvery green leaves
Apple Green	USA	heirloom American variety that produces round green eggplants, fruit matures for seed, the variety actually turns a cream yellow, light producer.
Louisiana Long Green	USA	produces long, thin, banana shaped, lime-green skinned eggplant fruits up to 8 inches in length with a sweet and mild tasting cream coloured flesh and matures in about 100 days.

Att.V Clone lines in Summer Experiment 2002

Eggplant genotype	Clone line	Replica	Eggplant genotype	Clone line	Replica
"Adona" (control)	6	4	"Dok"	4	1
	12	3		6	2
Total plant number "Adona"		7		8	2
"1507"	3	1		12	2
	5	2		13	2
	7	2		14	2
	8	2		15	4
	10	1		16	2
	16	1		19	2
	19	1		21	2
	25	1		33	2
	27	1	Total plant number "Dok"		23
	38	1	"Cao San"	2	2
Total plant number "1507"		13		6	2
"VASI-5"	2	2		12	2
	6	2		16	2
	13	2		17	2
	15	2		21	2
	16	2		23	2
	17	2		33	2
	35	1		35	2
Total plant number "VASI-5"		13	Total plant number "Cao San"		18

Att.VI DNA isolation Plant-Kit

100 mg plant material fine powder in the mortar	white column in a new tube + 500 µl buffer AW
+ 400 µl AP1 + 4 µl R-Nase (mix)	centrifugate 1 min. 8000 rpm
10 min 65°C incubate (mix 2-3 times) + 130 µl AP2	white column again in a new tube+ 500 µl buffer AW
necessarily 5 min on ice: fall out of proteins!!!	centrifugate 2 min. max. speed
centrifugate 5 min. max. speed	(column must be dry)
rest in lila column	+ 100 µl previously warmed AE buffer at 65°C –wait 5 min.-
centrifugate 2 min. 13000 rpm	centrifugate 1 min. 8000 rpm {first elution}
rest in new tube (meassure with pipette)	+ 100 µl previously warmed AE buffer at 65°C –wait 5 min.-
rest + 0,5 volumen AP3 + double alcohol (mix with pipette)	centrifugate 1 min. 8000 rpm {second elution}
650 µl in white column	DNA READY!!!
centrifugate 1 min. 8000 rpm	Electrophoresis (80V) for 20 min. and photograph under UV light to check DNA presence
rest again in white column	
centrifugate 1 min. 8000 rpm	

Att.VII EU marketing standards

Products subject to EU marketing standards

Fruits: apples, apricots, avocados, cherries, clementines, grapes, lemons, mandarins, melons, nectarines, oranges, peaches, pears, plums, satsumas, strawberries, water melons

Nuts: hazelnuts in shell, walnuts in shell

Vegetables: artichokes, asparagus, **aubergines**, beans, Brussels sprouts, cabbage, carrots, cauliflower, celery, cucumbers, garlic, iceberg lettuce, leeks, lettuce and endives, mushrooms (cultivated), onions, peas, spinach, sweet peppers, tomatoes, witloof, chicory. Mixed packages containing at least one of the products listed above.

I. DEFINITION OF PRODUCE

This standard applies to aubergines, fruit of the varieties (cultivars) grown from *Solanum melongena* L. var. esculentum, insanum and ovigerum, to be supplied fresh to the consumer, aubergines for industrial processing being excluded.

According to their shape, a distinction is made between:

- elongated aubergines,
- globus aubergines.

II. PROVISIONS CONCERNING QUALITY

The purpose of the standard is to define the quality requirements of aubergines after preparation and packaging.

A. Minimum requirements

In all classes, subject to the special provisions for each class and the tolerances allowed, the aubergines must be:

whole; fresh in appearance; firm; sound, that is produce affected by rotting or deterioration such as to make it unfit for consumption is excluded; clean; practically free of any visible foreign matter; provided with calyx and peduncle which may be slightly damaged; sufficiently developed, without their flesh being fibrous or woody and without over development of seeds (subject to the special provisions for Class III); free of abnormal external moisture; free of any foreign smell and/or taste.

The development and condition of the aubergines must be such as to enable them to:

withstand transport and handling; arrive in satisfactory condition at the place of destination.

B. Classification

Aubergines are classified into three classes defined below:

Class I

Aubergines in this class must be of good quality and must possess the characteristics of the variety. They must also be practically free from sunburn.

They may, however, show the following slight defects provided that these do not impair the general appearance, quality, conservation or presentation of the produce:

slight defect of shape; slight discolouration at the base; slight bruising and/or slight healed cracks provided that they do not cover more than 3 cm2.

Class II

This class comprises aubergines which do not qualify for inclusion in Class I, but satisfy the minimum requirements specified above. Provided they retain their essential characteristics of quality and presentation, they may show the following defects:

defects of shape;defects of colouring; slight sun-scorched provided it does not cover more than 4 cm2; slight dry superficial defects provided that they do not cover more than 4 cm2.

Class III (*)

This class comprises aubergines which do not qualify for inclusion in the higher classes but satisfy the requirements for

Class II.

However, they may:

be slightly fibrous; show considerable seed development; show sun-scorched provided it does not cover more than 6 cm2; show healed superficial defects provided that they do not cover more than 6 cm2.

III. PROVISIONS CONCERNING SIZING

The sizing of aubergines is determined:

- either by the maximum diameter of the equatorial section on the longitudinal axis,
- or by weight.

A. For sizing by diameter, the minimum diameter is fixed at 40 mm for elongated aubergines and 70 mm for globus aubergines.

The difference between the smallest and largest aubergines in the same package must not exceed:

- 20 mm for elongated aubergines,
- 25 mm for globus aubergines.

B. For sizing by weight, the minimum weight is fixed at 100 grams. The following scale must be complied with:

- 100 to 300 grams with a maximum difference of 75 grams between the smallest and largest aubergines in the same package,
- 300 to 500 grams with a maximum difference of 100 grams between the smallest and largest aubergines in the same package,
- above 500 grams with a maximum difference of 250 grams between the smallest and largest aubergines in the same package.

Compliance with the sizing scale is compulsory for Class I. In addition, elongated aubergines must have a minimum length, excluding peduncle, of 80 mm.

IV. PROVISIONS CONCERNING TOLERANCES

Tolerances in respect of quality and size are allowed in each package for produce not satisfying the requirements of the class indicated.

A. Quality tolerances

Class I

10 % by number or weight of aubergines not satisfying the requirements for the class, but meeting those for Class II or, exceptionally, coming within the tolerances for that class.

Class II

10 % by number or weight of aubergines satisfying neither the requirements for the class nor the minimum requirements, with the exception of produce affected by rotting, marked bruising, unhealed cracks or any other deterioration rendering it unfit for consumption.

Class III

15 % by number or weight of aubergines satisfying neither the requirements for the class nor the minimum requirements, with the exception of produce affected by rotting, marked bruising, unhealed cracks or any other deterioration rendering it unfit for consumption.

*Additional class as provided for in Article 2 (1) of Regulation (EEC) No 1035/72. The application of this

quality class or some of its requirements shall be subject to a decision to be taken under Article 4 (1) of the same Regulation.

B. Size tolerances

Class I

10 % by number or weight of aubergines conforming to the size immediately below or above that specified on the package.

Classes II and III

10 % by number or weight of aubergines not conforming to the minimum size. In any case, the tolerance is not applicable to aubergines the diameter of which is more than 5 mm below the minimum diameter or, in the case of sizing weight, to aubergines weighing less than 90 grams.

V. PROVISIONS CONCERNING PRESENTATION

A. Uniformity

The contents of each package should be uniform and contain only aubergines of the same origin, commercial type, quality and size (where sizing is compulsory) and appreciably the same degree of development and colouring.

In the case of Class III, it is enough that the origin and commercial type are uniform.

Elongated aubergines packed in the same package must be sufficiently uniform as regards length. The visible part of the contents of each package must be representative of the entire contents.

B. Packaging

The aubergines must be packed in such a way as to ensure proper protection of the produce.

The materials used inside the package must be new, clean and of a quality such as to avoid causing any external or internal damage to the produce. The use of materials, and particularly of paper or stamps, bearing trade specifications is allowed, provided that the printing or labelling has been done with a non-toxic ink or glue. The packages must be free from all foreign matter.

VI. PROVISIONS CONCERNING MARKING

Each package must bear the following particulars, in letters grouped on the same side, legibly and indelibly marked and visible from the outside:

A. Identification

- >PIC FILE= "T0035458">

B. Nature of produce:

"aubergines", if the contents are not visible from the outside; name of variety (optional).

C. Origin of produce:

country of origin; district where grown or national, regional or local place name (optional).

D. Commercial specifications

class; size (where applicable) expressed:

- either by maximum and minimum diameters, when sizing is by diameter,
- or by maximum and minimum weights, when sizing is by weight.

E. Official control mark (optional)